ARCHIVES OF PATHOLOGY

VOLUME 19 JANUARY 1935 NUMBER 1

PLASTIC STUDIES IN ABNORMAL RENAL ARCHITECTURE

III. THE AGLOMERULAR NEPHRONS OF TERMINAL HEMORRHAGIC BRIGHT'S DISEASE

JEAN OLIVER, M.D.
AND
ANN SEAWARD LUEY, A.B.
BROOKLYN

In a classic description of the progress of the destructive lesions in chronic glomerular nephritis there is always found the statement that obliteration of the glomerulus by the inflammatory reaction is followed by collapse and atrophy of its tubule. The integrity of the nephron is thus made dependent on adequacy of glomerular function, a concept which has a theoretical advantage since it explains the widespread effects noted in kidneys whose glomeruli are abnormal without shifting the emphasis from these relatively small elements that are presumed to play the primary and essential rôle in the disease.

It was the pathologic anatomist who established this conclusion, but if the method which was used for its foundation is examined it may be wondered whether that method was entirely adequate to the difficulty of the problem. In the study of a histologic section it is manifestly impossible to do much more than hazard a guess concerning the exact structural relations between the cross-sections of tubules and the scattered glomeruli, so that it seems probable that the morphologist has been forced to call on theoretical supposition as to what the effects of glomerular obliteration may be, and with this reasoned assumption piece out his fragmentary objective findings.

The exact line of reasoning behind the conclusion that the tubule must undergo an atrophy of inactivity when the glomerulus is destroyed is difficult to reconstruct in this day of conflicting theories of renal function. A simple statement of it might be that the water of the urine is derived from the activity of the glomerulus, and that the tubule collapses as a result of a drying up of this source. But the newer physiology gives pause to the ready assumption of so simple a chain of cause and effect. In any case the morphologist has no need to bolster his opinion by such

This work was done with the support of the Josiah Macy Jr. Foundation.

From the Hoagland Laboratory, the Department of Pathology, the Long Island College of Medicine.

borrowed support; his own means are adequate to an objective solution of a problem that is inherently structural, not functional. Two very old histologic methods have demonstrated that a tubule may persist in hypertrophic form when its glomerulus is reduced to an almost bloodless mass of collagen,[1] that the disruptive processes of disease may cut off tubules from glomeruli, and that these isolated tubules not only survive but maintain evidence of vital, progressive changes.[2]

The present article gives a detailed description of such aglomerular tubules and discusses the significance of the aglomerular nephron.

TECHNIC AND MATERIAL

The two methods of this study were plastic reconstruction of the abnormal elements by the Born wax plate method and isolation of the actual structures by microdissection from material macerated in strong hydrochloric acid. The details of these technical procedures have been previously described.[3]

Both of the methods make possible a plastic three dimensional study of the morphologic units. Each has its own peculiar value, for each not only confirms the other, but supplements the other where it is lacking. The chief advantage of the model is that it maintains in fixed form the relations of the elements as they existed in the tissues. Dissection, on the other hand, allows the study of a large number of examples and shows more clearly the finer details of their external structure.

The material studied consisted of kidneys presenting typical terminal hemorrhagic Bright's disease (chronic glomerular nephritis) taken in a series of cases the clinical and pathologic details of which have been described.[4] Since the individual case histories and pathologic observations have no especial bearing on our immediate problem, these have been omitted for the sake of brevity but may be obtained, if desired, by means of references appended to the descriptions of the figures.

MANNER OF ORIGIN AND CONSEQUENT STRUCTURE OF THE AGLOMERULAR TUBULE

If the deeper layers of the cortex and the outer stripe of the outer zone of the medulla of a kidney that shows the more severe lesions of terminal Bright's disease are dissected beneath the microscope, among the large hypertrophied and hyperplastic terminal portions of the proximal convolutions are found evidences of tubular disruption.[2] The accumulation of inflammatory exudate and granulation tissue produces an

1. Oliver, J., and Lund, E.: Arch. Path. **15**:755, 1933.
2. Oliver, J., and Luey, A. S.: Arch. Path. **18**:777, 1934.
3. Oliver and Lund.[1] Oliver and Luey.[2]
4. Addie, T., and Oliver, J.: The Renal Lesion in Bright's Disease, New York, Paul B. Hoeber, Inc., 1931.

irregularly distributed pressure that distorts the tubular elements lying in the involved areas. When, in the later stages of the reaction, shrinkage of collagen adds its disruptive force, an actual interruption of the tubule may result. In many cases the interruptions are multiple, and minute cysts that approximate in size the diameter of the original tube are found scattered widely through the tissue. If it is the hypertrophied terminal portions of the proximal convolutions that are affected, irregular vesicles are produced whose peculiarly contoured walls preserve very exactly the configuration of the tubule from which they arose. Often these vesicles may be found in a linear arrangement that clearly shows the original course of the former tubule (fig. 1, *1*).

The vesicles are enclosed structures and so remain as inert evidences of the destructive processes. But after fragmentation of a tubule has occurred, its distal portion continues on its course. At times a considerable part of the hypertrophied terminal segment may thus persist intact as a short bulky "convoluted tubule" which, transformed into the narrow segment of Henle's loop, passes through its customary bend and ascends to form the distal limb in an entirely normal manner (fig. 1, *1*).

These effects of disruption are seen in irregular patches that lie scattered among tissues where the tubules have remained intact. In the uninvolved areas one may search in vain for fragmented structures, but if a proper region is located by the finding of vesicles, a further search almost certainly results in the discovery of a considerable number of interrupted, that is, aglomerular tubules. They are of all sizes, from long tortuous and kinked hyperplastic or hypertrophied tubules that represent the greater part of a terminal portion of a proximal convolution (fig. 1, *2*) to short stubby remnants of its very tip (fig. 1, *3 and 4*). In either case the fragile and tenuous narrow segment of Henle's loop may be found, normal in appearance and in the direction of its course. If it is somewhat dilated, this dilatation is proportional and therefore proper to the degree of distention that exists in the remnant of proximal convolution from which it arises.

In the aglomerular tubules just described, hypertrophic thickening of the walls and hyperplastic kinking are combined with dilatation of the lumen of the tubule. In others an almost pure hypertrophy of the wall with no significant dilatation is noted. Such tubules appear dense and solid, with no external evidence of a lumen. Associated with the hypertrophy is always a certain amount of hyperplastic kinking (fig. 1, *5* and *6*).

Other aglomerular tubules show little thickening of their walls, but are merely dilated (fig. 1, *7*, *8* and *9*). It must be remembered that the absence of an absolute increase in the thickness of the walls of a dilated tubule does not indicate the lack of a hypertrophic or hyperplastic process in its constituent cells. In such examples of extreme

Explanation of Figure 1.

Fig. 1.—All the representations show the specimens under a magnification of × 15. The corresponding case histories and pathologic observations may be obtained elsewhere (Addis and Oliver [4]). Explanations of the drawings follow:

1. A hyperplastic and dilated terminal portion of a proximal convoluted tubule that has been cut into three segments. The lower segment, beginning as a blind end, passes into the narrow portion of Henle's loop. Addis and Oliver,[4] case 6, p. 197.

2. An aglomerular blind tubule formed by detachment of a hyperplastic and hypertrophic terminal portion of a proximal convolution. It passes into the narrow limb of Henle's loop, which in turn widens into the broad ascending portion. Addis and Oliver,[4] case 6.

3. A blind tubule, hypertrophied and hyperplastic, derived from the tip of the terminal segment of the proximal convolution, with the first portion of the narrow limb of Henle's loop. Addis and Oliver,[4] case 6.

4. Another similar very short blind aglomerular tubule showing hypertrophy, hyperplasia and dilatation. Addis and Oliver,[4] case 6.

5. A blind tubule showing almost pure hypertrophy and hyperplastic kinking with little evidence of dilatation. Addis and Oliver,[4] case 6.

6. A similar solidly hypertrophied aglomerular tubule with the beginning portion of the narrow limb. Addis and Oliver,[4] case 6.

7. A short blind tubule with hyperplastic kinking and marked dilatation. Addis and Oliver,[4] case 6.

8. Another dilated blind tubule. Addis and Oliver,[4] case 24, p. 268.

9. A blind tubule showing a combination of hyperplastic kinking and irregular dilatation. Addis and Oliver,[4] case 6.

10. A short blind tubule that shows considerable dilatation. Addis and Oliver,[4] case 24.

11. An aglomerular tubule with irregular dilatation in its lower portion. Addis and Oliver,[4] case 24.

12. A blind tubule showing extreme dilatation. Even the first part of the narrow limb is distended. Addis and Oliver,[4] case 24.

13. A long blind tubule with dilatation and fatty deposit. Addis and Oliver,[4] case 24.

14. A blind tubule showing combined dilatation and hyperplastic kinking and considerable fatty change. Addis and Oliver, [4] case 24.

15. Another dilated blind tubule with fatty deposits in its walls. Addis and Oliver,[4] case 24.

16. An aglomerular tubule showing irregular dilatation and hyperplastic kinking. Note the pointed filamentous origin at the blind end. Addis and Oliver,[4] case 24.

17. Another similar tubule showing hyperplasia and irregular dilatation with the narrow and broad portions of Henle's loop. The blind end of this tubule is also drawn to a sharp point. Addis and Oliver,[4] case 24.

18. A complete aglomerular nephron. The tubule begins with a tapering blind end and passes downward with marked hypertrophy, hyperplastic kinking and irregular dilatation. Then follow the narrow and the broad portion of Henle's loop, which passes into the distal convolution to join the collecting tubule. Note that there is no obstruction in the course of the tubule. Compare with the complete normal nephron shown in figure 2 in the previous study of this series. Addis and Oliver,[4] case 24.

19. Another complete short loop aglomerular nephron. Above and to the right is seen the glomerulus cluster of the proximal convoluted tubule. Below, the terminal portion of the proximal convoluted tubule has been separated from it. The aglomerular nephron begins with a rounded blind end, descends to form the loop, then ascends through the narrow and broad portions to form the distal convolution and passes by the connecting piece into the peripheral collecting tubule. Addis and Oliver,[4] case 32, p. 325.

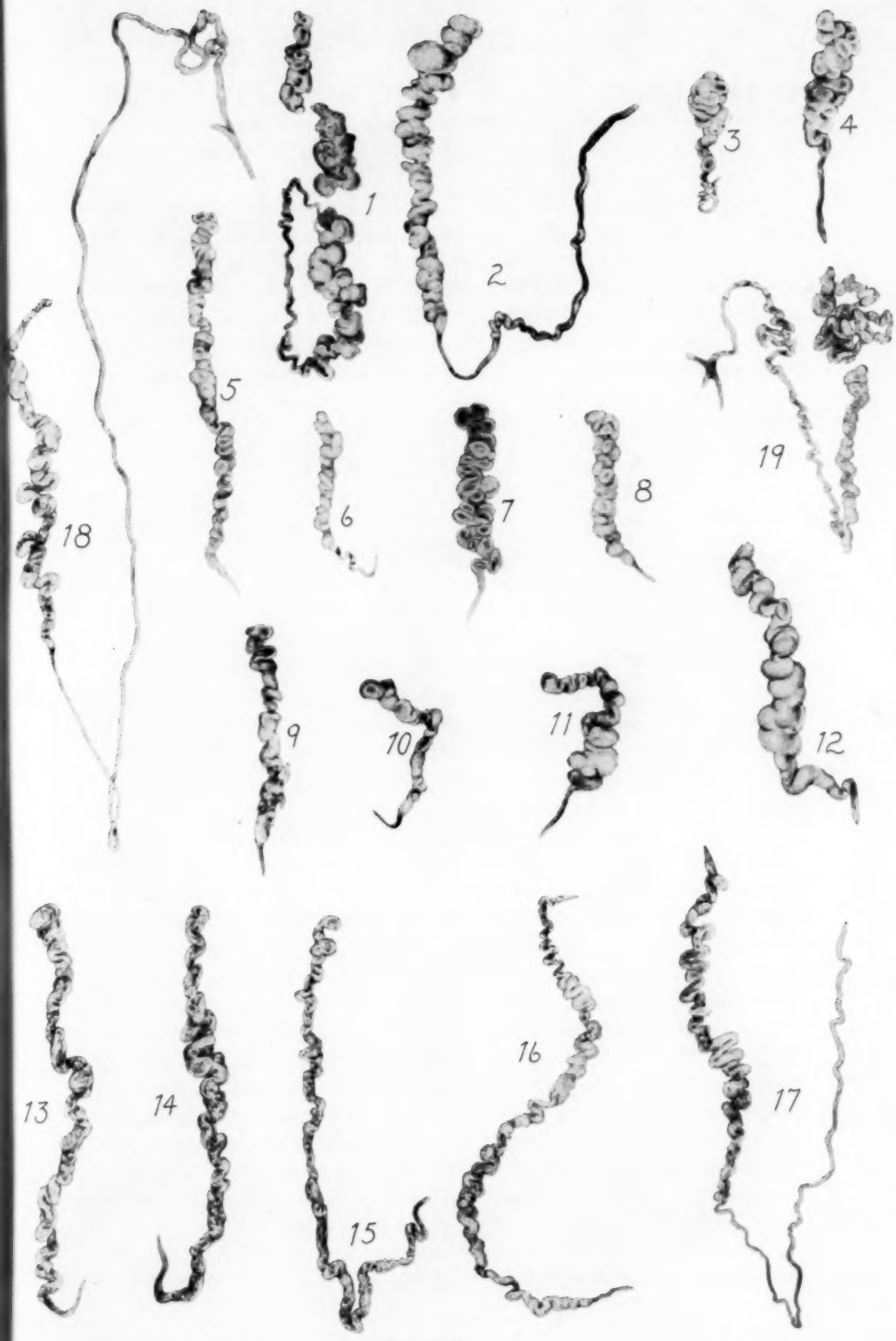


Figure 1.

dilatation as are shown in figure 1, *10, 11* and *12,* in which the walls are no thicker than those of a normal tubule, it is plain that there must be a great increase in either the size or the number of the cells forming this greatly increased extent of tissue.

The changes in the walls of the aglomerular tubules so far described, namely, hypertrophy and hyperplasia, are progressive. Regressive changes also occur of a nature and distribution similar to those seen in the intact nephrons of the abnormal kidney.[2] Cloudy swelling of the tubule cells is present, though not easily observable in an examination of the tubule as a whole, but the presence of fat droplets is strikingly apparent because of their bright refractile nature. Dilated aglomerular tubules are especially likely to show them in considerable amount (fig. 1, *13, 14* and *15*).

ACTUALITY AND MORPHOLOGIC COMPLETENESS OF THE AGLOMERULAR NEPHRON

The first question that arises in the mind of the observer of these unusual structures is whether they may not be fragments that have resulted from the technical manipulation. Dissection with relatively coarse needles of objects so delicate and fragile is at best a difficult procedure, and the fact that less than a half dozen complete mammalian nephrons have been illustrated in the literature is evidence that fracture is the rule even with the most careful dissection.

In the isolation of suspected aglomerular tubules extreme care was taken to locate first the blind end of the tubule and then, as the gentle manipulation proceeded, to keep it in view. An examination of the blind ends shows two typical forms. In one the end is prominent and projects in a rounded knob to form a short finger-like process (fig. 1, *5* and *6*). In the other form the tubule gradually tapers and ends in a tenuous filament (fig. 1, *16* and *17*). These blind ends may be turned with the needles and inspected from all sides without an interruption being shown in their smooth surface. In the great majority of cases a casual glance at the torn end of an artefact is sufficient cause for its rejection. However, a kinked coil of a fractured hyperplastic tubule may at times curl on itself and bury its fragmented end, so that in case of uncertainty the tubule was removed from the water in which it was suspended, placed on a slide beneath a cover slip and examined by transmitted light at high magnification. If the pressure of the cover slip had not burst the tubule, a mischance which at least favors a conservative decision of the matter, the blind end was plainly seen completely enclosed by an intact layer of epithelium. Several of these blind ends from tubules previously illustrated are shown in figure 2.

The solution of our first uncertainty by the demonstration that the interruption of the tubule at its proximal end is a bona fide result of

the pathologic processes of the disease sharpens, perhaps, the point of a second criticism. If the nephron was in fact interrupted by the disease in one part of its course, how can we feel certain that the aglomerular tubules so far presented have not been interrupted again in some part of the long reach that lies before the collecting tubule? Are they perhaps only special cases of the inert vesicular fragments that we have previously described? If this is the case, the aglomerular tubule can have little vital significance and hardly merits the term "aglomerular nephron." A demonstration of continuity is therefore required.

The direct and objective demonstration of this continuity is the isolation of an aglomerular nephron intact from its origin at the point of

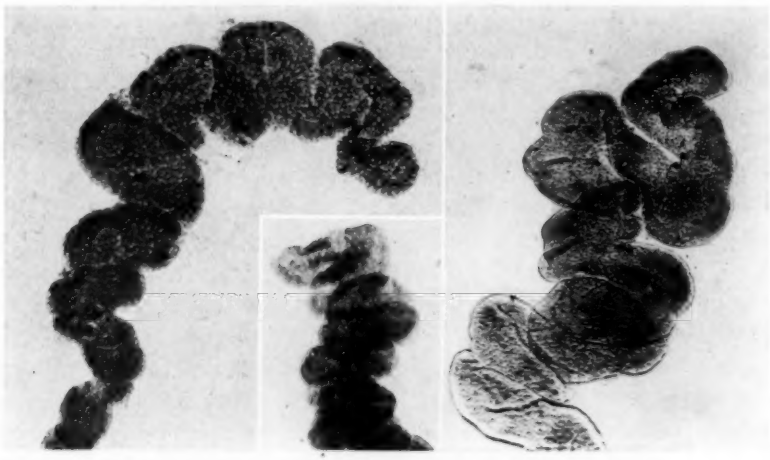


Fig. 2.—Photomicrographs of the blind ends of unstained typical aglomerular tubules. Note the smooth rounded contour of their termination and the absence of fracture. × 100.

interruption, which lies in the terminal portion of the proximal convolution, through the narrow part of Henle's loop and its bend, up the broad ascending limb to the distal convolution and by the connecting piece into the peripheral collecting tubule. As we have said, only a few such complete nephrons have been successfully isolated even in the normal kidney where no complications of the procedure exist. The most serious difficulty in the attempted isolation of an aglomerular nephron was the finding of a proper short loop example. Not infrequently considerable time was spent in careful dissection only to find that the nephron was a long loop and the successful isolation of it impossible, owing to the extreme length of the narrow portion of Henle's loop, or, as the dissection proceeded, it became evident that the nephron was not an aglomerular one.

Two complete intact aglomerular nephrons are shown in figure 1, *18* and *19*. In *18* the terminal segment of the proximal convolution has been reduced to a thin filament which grows progressively thicker until it finally becomes greatly enlarged as a result of hypertrophy and some dilatation. Hyperplastic kinking is also present, so that the tubule resembles a common type of terminal segment seen in the intact abnormal nephron. This remnant of the proximal convolution passes into the narrow limb of Henle's loop; the broad portion is formed, turns through the bend, then ascends to extend through a normal distal convolution and reaches by way of its connecting piece the collecting tubule. The point of entrance of another nephron identifies the latter. It will be noted by a comparison with figure 2 of the preceding study in this series [2] that the complete nephron distal to its aglomerular proximal convolution is entirely normal. There is no plugging of any portion of it, and no dilatation of even the extremely delicate and thin-walled narrow segment of Henle's loop.

In figure 1, *19* not only the aglomerular nephron is shown but also the proximal convolution of the original unit from which it arose. From the glomerulus, about twice its normal size, arises the periglomerular cluster of the coils of the proximal convolution. These are definitely dilated, and their walls are sprinkled with fine fat droplets. Near the lower part of this cluster arises the separated terminal portion of the proximal convolution. It begins as a knoblike projecting end and continues in the form of a typical hypertrophic and hyperplastic kinked spiral segment. The bend of the loop is situated at its immediate end, an abnormality very common in the nephron of terminal Bright's disease,[2] and from there on the course of the tubule through the distal convolution into the collecting tubule is entirely normal.

As another evidence of continuity, attempts were made, with no success, to inject a fluid into the aglomerular nephrons from the pelvis of the kidney. In fact, owing to the plugging of the collecting tubules with débris and casts,[2] not even these tubes could be regularly filled with the injection fluid.

RELATIONS OF THE AGLOMERULAR NEPHRON TO NEIGHBORING STRUCTURES

The method of dissection, though it demonstrates in a most striking manner the details of the general structure of the aglomerular nephrons, is inadequate for the demonstration of the topographic relationship that the interrupted portion bears to the surrounding elements of the tissues, since the essential procedure in the method is the removal of the object from its natural surroundings. In figure 1, *19*, it is true, a fortunate chance allowed the identification of the proximal part of the nephron from which the aglomerular portion was derived. But it is concerning

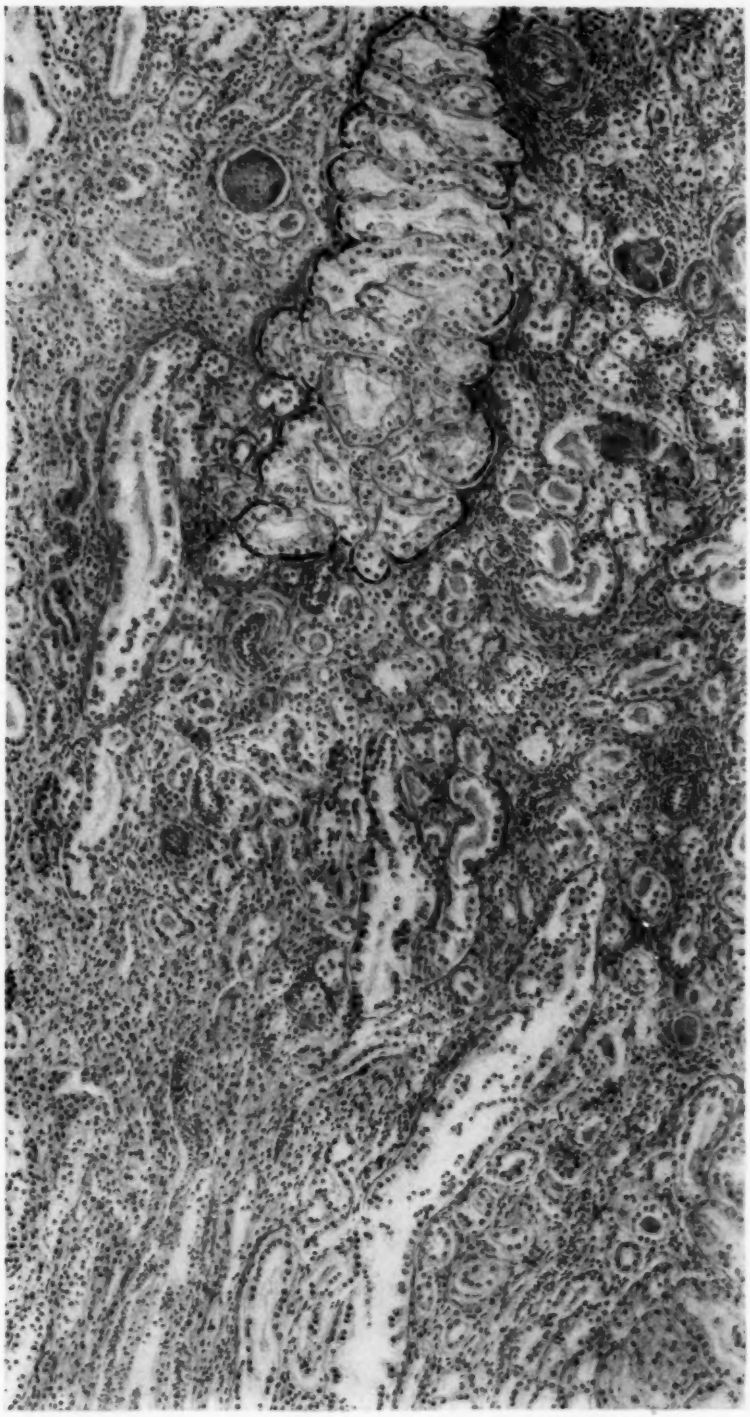


Fig. 3.—Section from the outer stripe of the outer zone of the medulla passing through the center of the aglomerular tubule shown in the model of figure 5 and stereogram 1. The sections that are included in the model have been outlined with black. They form an island surrounded by inflammatory scar tissue in which are scattered atrophic and dilated tubules. Note the hypertrophic, well preserved cells that line the blind tubule. × 100. Addis and Oliver,[4] case 32, p. 325.

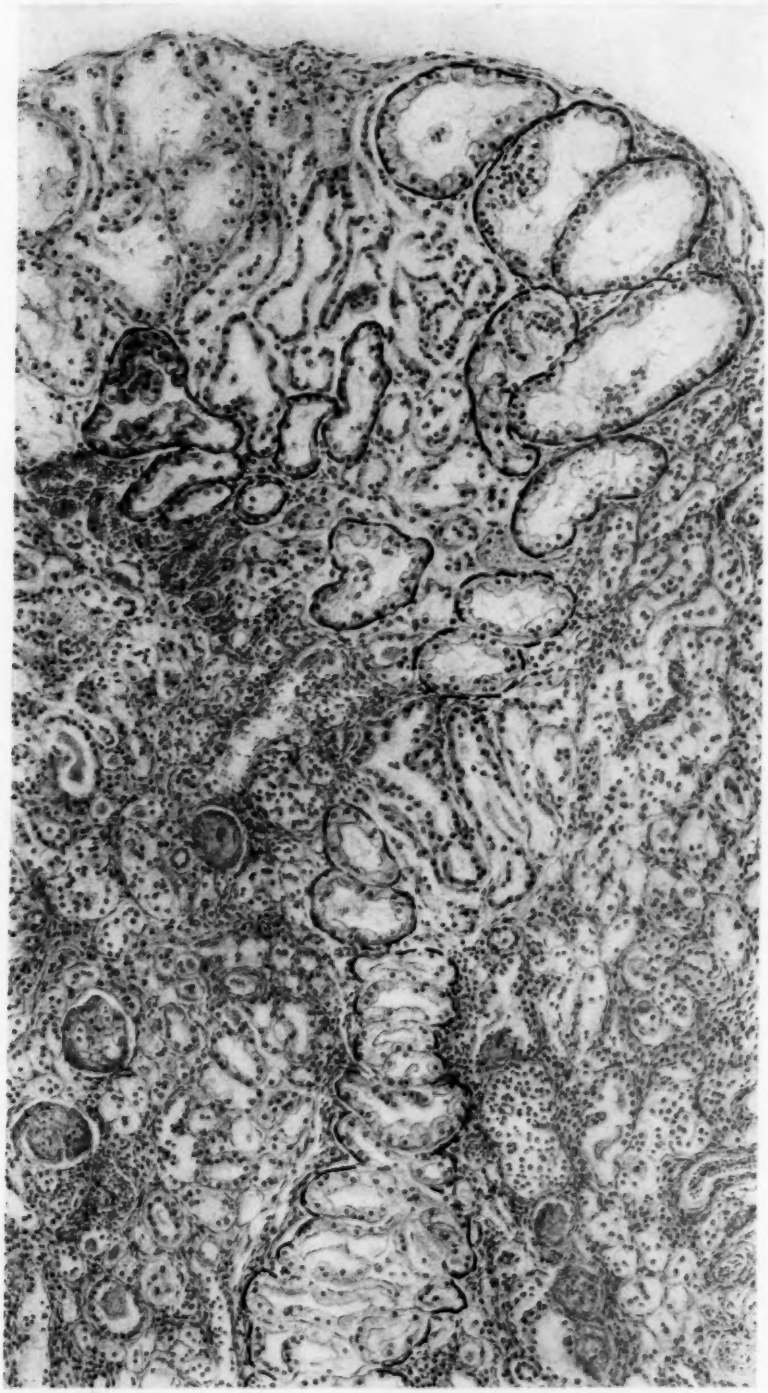


Fig. 4.—The cortex immediately above the area shown in figure 3. Below are seen sections of the upper portion of the blind tubule. In the upper third of the section and to the left is the glomerulus with the origin of the proximal convolution surrounded by atrophic as well as greatly dilated tubules. The sections which appear in the model of figure 6 and stereogram 3 have been outlined in black. Note the dense scar that surrounds the glomerulus and the group of tubules. × 100.

the vascular relations in particular that information is desired, and satisfactory observations are entirely impossible in dissected material. Recourse was therefore had to the method of reconstruction for these supplemental data. Moreover, the method of reconstruction from serial sections affords, as a by-product, information of the greatest value, for it makes possible a study in histologic sections of the epithelium that forms the aglomerular tubule. Such information will be essential to a final judgment as to the adequacy of these nephrons.

A kidney was chosen that was known to contain aglomerular tubules in considerable number. In the histologic section one cannot determine

Fig. 5.—The aglomerular tubule of the model shown in stereogram 1. The blind end is seen at its upper end. × 50.

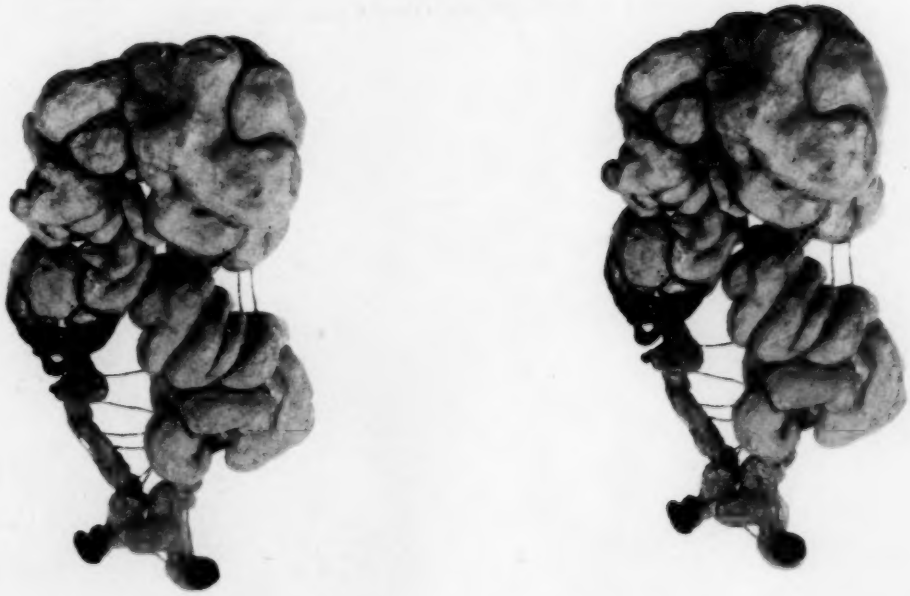
certainly whether a tubule has been interrupted or not, but a fair guess as to the likelihood of such an occurrence can be made in a case that has been thoroughly studied by microdissection. An example was chosen and drawings at a magnification of 200 were made in the usual manner of the method. The tubule was then traced, and if it showed a normal continuous course into the periglomerular cluster of the proximal convolution, the procedure was abandoned and another chosen.

In figure 3 and in the lower half of figure 4 is shown a section passing through the aglomerular tubule of the first model. The marked hyperplastic kinking of it can be recognized even in the section. Its lumen is not greatly dilated, and its cells are normal both in their type

Stereogram 1.—An aglomerular tubule composed of a hypertrophic and hyperplastic terminal portion of the proximal convolution. Above, the knotlike blind end is seen; below, the passage into the narrow portion of Henle's loop is evident. × 35. For this and the following stereograms the designation of the various structures may be obtained from the corresponding text figure. Addis and Oliver,[4] case 32, p. 325.

Stereogram 2.—The glomerulus and periglomerular cluster of the proximal convolution from which the blind tubule of the previous model was derived. Note Ludwig's vessel projecting from the afferent vessel. × 35.

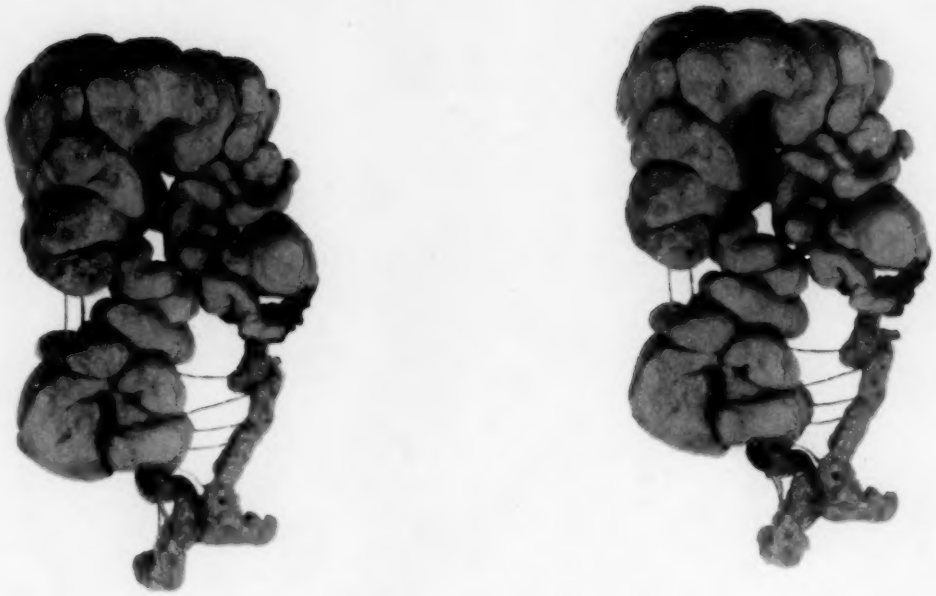
Stereogram 1



Stereogram 2

Stereogram 3. —The obverse side of the same model showing the origin of the proximal convoluted tubule from the glomerulus and the blind end of the interrupted proximal convolution. × 35.

Stereogram 4.—A fibrosed glomerulus with the atrophic remnant of the proximal convoluted tubule. × 35. Addis and Oliver,[4] case 15, p. 238.



Stereogram 3

Stereogram 4

Stereogram 5.—The aglomerular tubule lying in the vicinity of the atubular glomerulus of stereogram 4. × 35.

Stereogram 6.—The atubular glomerulus and the aglomerular tubule as they lay in the tissues. Note the two blind ends. × 35.

Stereogram 5

Stereogram 6

and in their state of preservation. They are in fact larger than the cells of the corresponding portion of the normal convoluted tubule. The cross-section of the tubule forms an isolated island that lies completely surrounded by inflammatory tissue and atrophied tubules. In this scar tissue there is a certain amount of collagen, but it is still heavily infiltrated with leukocytes that include both lymphocytes and polymorphonuclears.

The model (fig. 5 and stereogram 1) shows that the structure is a typical undilated hyperplastically kinked hypertrophic terminal spiral segment. Below, its abrupt transition into the narrow segment of Henle's loop is seen. Above, it doubles on itself in a knotlike cluster of coils and ends blindly. In its general configuration it resembles the actual isolated specimen of figure 1, *16*.

The relation of the aglomerular tubule to the surrounding scar tissue and the normal characteristics of its constituent cells have been seen in the sections from which the model was prepared. Its relation to the proximal portion of the original nephron from which it was separated can also be demonstrated in the sections. In the immediate neighborhood of the blind end of the tubule is situated a group of cross-sections of a proximal convolution that surrounds a glomerulus (fig. 4). Some of the cross-sections, especially those that lie near the upper end of the reconstructed blind tubule, are greatly dilated, while to the left, directly in contact with the glomerulus, they are surrounded by the inflammatory scar tissue, which infiltrates between them, and are consequently distorted and atrophied. The section through the glomerulus passes through the exit of the tubule, but only tangentially cut portions of the tuft are present. In other sections this structure showed a heavy infiltration with leukocytes and fibrosis with collapse of the capillaries.

This glomerulus and its surrounding tubule along with another glomerulus and its cluster form a "granule" which projects on the surface of the kidney. In section the granule is limited on each side by inflammatory tissue that, extending deep into the cortex, encloses in a cone-shaped area the two fragments of the nephron which appear in our reconstructions.

The second model in its front view (fig. 6 and stereograms 2 and 3) shows the glomerulus at the left of the clustered tubules almost completely buried in the mass of their coils. The origin of the proximal convolution is visible in the posterior view where the glomerulus lies free. The first loops are extremely tortuous and markedly atrophied, but as they ascend they gradually increase in diameter until, passing laterally, they become enormously dilated. In some portions they are even greater in diameter than the glomerulus. The dilated tubule now

descends (posterior view) and forms a second mass of convolutions. From this mass no tubule emerges to form a terminal spiral end-segment. In fact, the tubule ends in a blind pouchlike process that is embedded among the neighboring coils (posterior view, *x*).

The relation of the two portions of the proximal convolution, the glomerulus with its pericorpuscular cluster and the detached hyperplastically kinked terminal segment is shown in figure 7. All of the free space that lies between the two and all that lying between the masses of dilated tubular coils is to be imagined as filled with inflammatory scar tissue of the sort shown in figures 3 and 4. In this scar tissue are

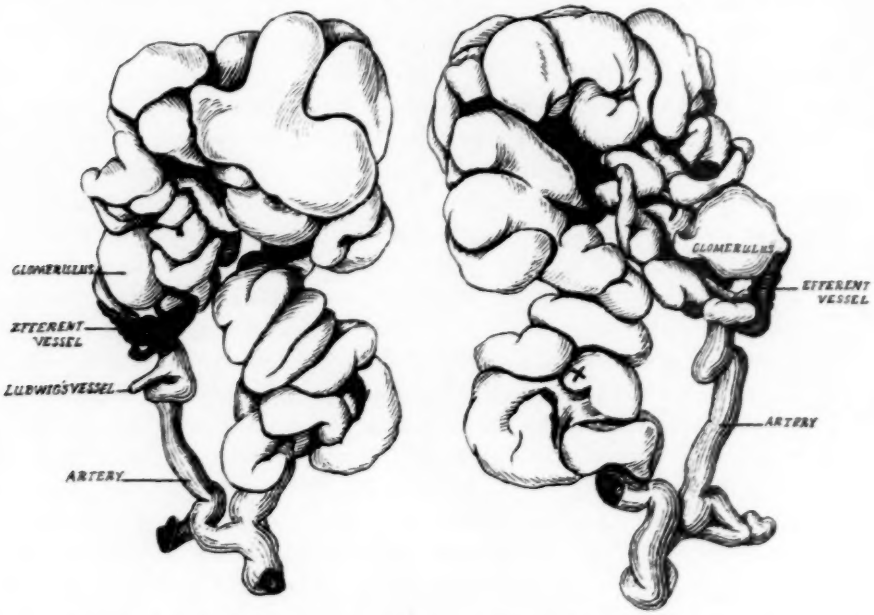


Fig. 6.—Anterior and posterior aspects of the model shown in stereograms 2 and 3. The proximal convolution ends blindly at *x*. × 50.

innumerable islands of atrophied epithelium and collapsed and atrophied tubules. It is impossible to identify any of these as the actual remnants of the connection which once existed between the two blind ends, but it is certain that among such fragments must lie the remains of the disrupted tubule, and the general relation between the two fragments of the original nephron is therefore evident. It is of interest that this relation is very similar to that observed in an actual dissected specimen (fig. 1, *19*).

Not only is the topography of the two separated portions of the original nephron shown by the models, but the condition of the vascular apparatus is presented. In figure 6 and stereograms 2 and 3, the inter-

lobular artery is shown breaking up into four terminal branches at the base of the lower cluster of tubules. One of these branches ascends directly to the glomerulus and enters it (anterior surface and stereogram 2) as the afferent arteriole. On the posterior surface (stereogram 3) is seen the exit of the efferent vessel, which almost immediately is lost in the intertubular plexus (anterior surface). As has been stated

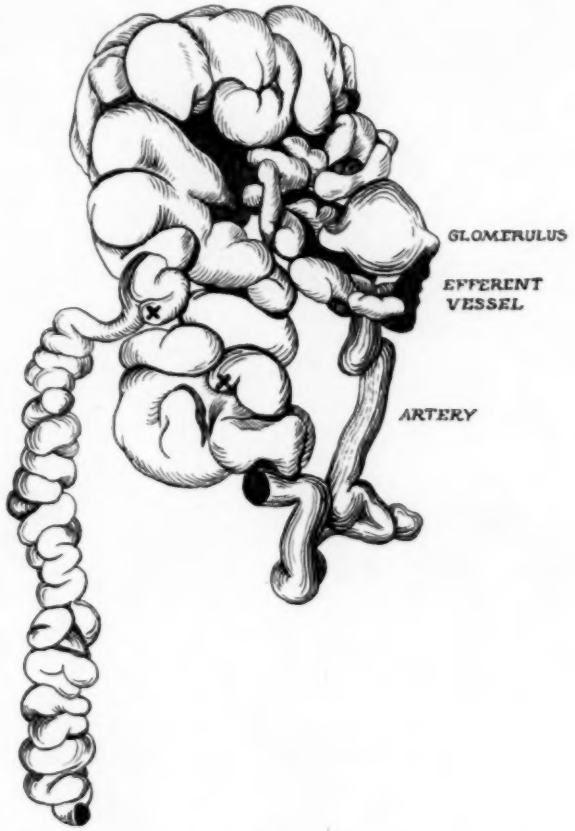


Fig. 7.—Relative positions occupied in the tissues by the structures shown in the models of stereograms 1 and 2. × 50.

in the description of the section through the tuft of the glomerulus, the capillaries, if not completely obliterated, were reduced at least to an occasional uncertain tortuous channel. That the intertubular capillaries were not, however, dependent for their supply on the patency of these narrow passages is evident by the recognition of Ludwig's vessel, a short branch that juts out from the afferent arteriole just below the efferent plexus (anterior view, stereogram 2). At its end is the junc-

tion with the intertubular system. The latter is not shown, as the exact reconstruction of tortuous capillaries is very inaccurate and of no special significance.

For the next model a glomerulus was chosen that was, as far as could be seen in the sections, entirely bloodless, since it had been transformed into a mass of cellular connective tissue by the inflammatory

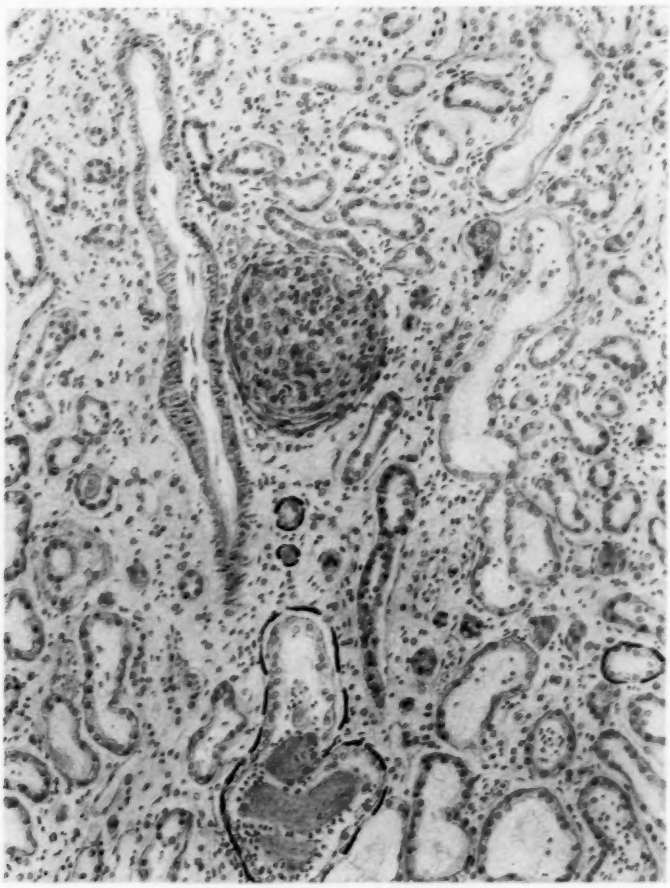


Fig. 8.—Section passing through the glomerulus and blood vessel of the models shown in figures 9 and 10 and stereograms 4 and 5 (Addis and Oliver,[4] case 15, p. 238). The two small tubules outlined in black are included in the atrophic remnant of the proximal convolution that is attached to the glomerulus. The sections through the two larger tubules are included in the model of the aglomerular tubule shown in stereogram 5. Note the dense scar tissue surrounding the glomerulus. The glomerular tuft is avascular. × 100.

process which had occurred within it (fig. 8). Moreover, it was surrounded by a dense fibrous inflammatory scar in which only small

atrophic tubules lay. Beside it was seen the irregularly thickened interlobular artery.

The reconstruction is shown in figure 9 and stereogram 4. From the thickened intertubular artery springs a short afferent arteriole which enters the glomerular body on its superior aspect. Adjacent to this point of entrance the efferent vessel emerges, stopping abruptly in the model, since it blends at once with an irregular capillary network that could not be followed.

The body of the glomerulus is small and distorted, and from its inferior margin arises the proximal convolution. This is a tortuous shrunken tubule, of perhaps one-third the normal diameter, which after

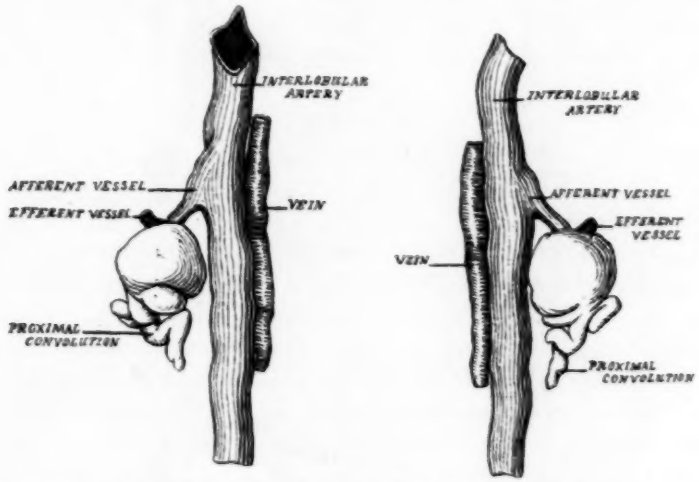


Fig. 9.—Anterior and posterior aspect of the model of the atubular glomerulus shown in stereogram 4. × 50. Addis and Oliver,[4] case 15.

a few feeble and irregular coils ends in a finger-like process. The structure, for all practical purposes, is therefore an atubular glomerulus.

In the periphery of the scar tissue that surrounds the glomerulus are a few scattered cross-sections of large convoluted tubules (fig. 8). Their lumens are dilated, and some contain débris, but the epithelium is everywhere intact and essentially normal in type. These were drawn and their course traced through the sections, and it was found that the reconstructed tubule was not attached to a glomerulus but began as a narrow blind end (fig. 10 and stereogram 5). Its origin is seen at *x* in the posterior view of the model (fig. 10 and stereogram 6) as a narrow irregularly constricted tubule that makes its way upward to the apex of the clustered mass. It then turns abruptly back on itself, passes through several irregular bends and then, forming a large loop

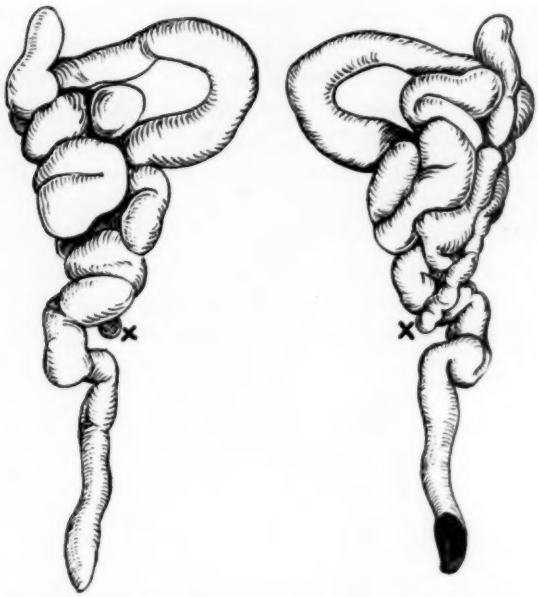


Fig. 10.—Anterior and posterior views of the aglomerular tubule of stereogram 5. Note the origin of the blind tubule at *x*. × 50. Addis and Oliver,[4] case 15.

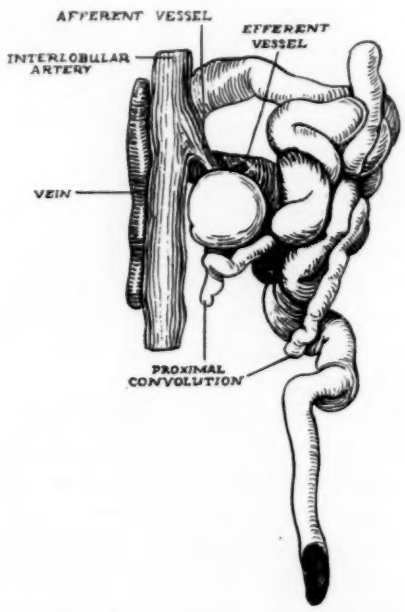


Fig. 11.—Relative positions in the tissues of the atubular glomerulus and the aglomerular tubule. Note the proximity of the two blind ends that in figure **8** are shown to be surrounded by dense scar tissue. × 50.

that projects laterally, descends in further secondary coils and passes into the relatively straight portion of the terminal segment.

One has, therefore, an atubular glomerulus and an aglomerular tubule lying within a mass of inflammatory scar tissue. The exact relation of the two as they lay in the tissues of the kidney is shown in figure 11 and stereogram 6. Again all free spaces are to be imagined as filled with the tissue of the inflammatory scar, and reference to figure 8 will show that in the region of the blind ends, lying in the model not a great distance from each other, are scattered masses of atrophic epithelium that can well be remnants of the disrupted tubule. In its general configuration, especially in its tapering filament-like origin, the reconstructed aglomerular tubule resembles the dissected specimen of figure 1, *18*. The advantage of the reconstruction is that the model allows the identification of the detached glomerulus.

COMMENT

Our earlier demonstration of the development, during the course of a glomerular nephritis, of hypertrophied tubules that in a functional sense may be considered as aglomerular is now supplemented by the finding of equally well preserved tubules that have no physical connection with glomeruli. Analogous aglomerular tubules have been recently described in experimental chronic uranium nephritis by MacNider,[5] so that there seems to be no doubt that destruction of the glomerulus by varied sorts of lesions may leave the tubule still structurally intact.

Certain of the facts brought forward in our description may now be emphasized because of their bearing on possible inferences concerning the functional significance of the aglomerular nephron.

Aglomerular tubules are not incomplete fragments of the original structures but may persist as complete aglomerular nephrons. Their continuity has been directly demonstrated from their origin in the proximal convolution through the loop of Henle and distal convolution into the collecting tubule. In the examples shown, there were no abnormalities in the distal portions of the tubule, such as obstruction or dilatation.

The aglomerular nephrons not only persist as complete structures, but maintain the structural peculiarities of the original unit, namely, hypertrophic and hyperplastic change. Moreover, they maintain these evidences of progressive growth under the most adverse conditions in areas where the processes of the disease are causing atrophy and destruction of other structures. A histologic examination of the cells of these enlarged tubules shows them to be increased in size, normal and well preserved in cytologic detail.

5. MacNider, William de B.: Proc. Soc. Exper. Biol. & Med. **31**:293, 1933.

Although the constituent cells of many of the aglomerular tubules are normally preserved, in others the same reactions to the abnormal conditions of the disease are found as in the tubules of the intact nephrons. The evidence of similarity of cellular response is seen in the regressive changes of cloudy swelling and fatty degeneration.

The aglomerular nephrons have a blood supply that is entirely adequate for maintenance and growth in spite of the obliteration of the capillary bed of the glomerular tuft. This compensatory blood supply is through the vessel of Ludwig which shunts the blood from the afferent arteriole directly into the intertubular capillary system.

The mechanism by which an adequate compensatory blood supply is established in the kidney of a patient with glomerular nephritis when the essential lesion has resulted in the occlusion of the primary capillary bed of the kidney is a problem that has taxed the ingenuity of pathologists ever since the early demonstration that the greater part of the blood reaching the tubules must first pass through the vessels of the tuft. A summary of the conflict of opinion in the matter is well illustrated by the discussion at the fifteenth meeting of the Deutsche pathologische Gesellshaft, at which Stoerck declared that the tubule "disappears" when the glomerular circulation is destroyed, because the blood supply to the glomerulus and its proximal convoluted tubule is a "unit" independent of anastomoses from neighboring intertubular networks. In glomerular nephritis, the kidney is maintained only by the "regeneration" of those units whose glomeruli have not been severely involved.[6] Marchand in opening the discussion doubted the absence of anastomoses between units, and felt that blood might well be derived from the contiguous capillaries. Aschoff shared this opinion and declared, moreover, that the disappearance of the tubules was due not to anemia but to an atrophy of inactivity. Gross cited the experiments of Lindeman, who occluded the glomerular capillaries by means of oil droplets without resulting tubular lesions. Kaiserling, taking an attitude between the two extremes, pointed out that a lack of tubular lesions might be due to the persistence of an occasional channel through the fibrous mass of the glomerulus, since forcible injection often shows the presence of such an unsuspected persisting path for the blood. Without reaching any concerted conclusion, therefore, Orth closed the matter with the pertinent exclamation that since the cells of even the most atrophied tubules are living—*"Da müssen sie doch noch ernährt sein!"* (Then must they still be nourished!)

How such nourishment might come about was definitely shown by Elise Dehoff.[7] Ludwig's vessel was demonstrated in reconstructions of

6. Stoerck, D.: Centralbl. f. allg. Path. u. path. Anat. (supp.) **23**:225, 1912.
7. Dehoff, E.: Virchows Arch. f. path. Anat. **228**:134, 1920.

the arteries and afferent vessels of the normal kidney, but the attempt to show their presence in kidneys affected with glomerular nephritis failed. Nevertheless since this demonstration in the normal kidney was made it has been tacitly assumed that the vascular lesion of the disease spares these branches and that they function in the maintenance of tubular nutrition.

In a former study [2] we showed the extent of the conversion that takes place in the terminal arterial circulation of the kidney as the disease progressively destroys glomerular tufts. The efferent vessels decrease in size and become difficult to find while the vessels of Ludwig become conversely enlarged and prominent and easy to demonstrate by dissection. The structural relation of these vessels also has been shown in the second model of this study. MacNider [5] has pointed out the possibility of the reestablishment of circulation in areas of fibrosis and glomerular destruction by the ingrowth of vessels from neighboring regions of the medulla and from cortical adhesions. Such a possibility must be admitted, though the failure of the therapeutic procedure of decapsulation, which in its earlier application was based on the latter part of this assumption, does not indicate that this mechanism can be adequate. A supply through Ludwig's vessel is more direct, and furthermore, the mechanism of its development is a remarkably efficient one, since it functions automatically. Closure of the capillary bed in the glomerulus by any means would at once open by hydrostatic pressure the by-pass of the shunting vessel, and the circulation to the tubules might therefore be scarcely interrupted.

Another peculiarity of the circulation to the aglomerular nephrons was pointed out in our former investigation.[2] Not only may the amount of blood reaching the tubules be adequate, but this blood must be peculiarly stimulating to progressive reactions because of the directness of its course to the tubules. It has not passed through the glomerular tuft but reaches the tubules still laden with those products requiring elimination that under normal conditions would have been removed by glomerular activity. The demands for functional response on the aglomerular tubule and the corresponding growth processes of hypertrophy and hyperplasia must therefore be abnormally great.

With these points emphasized, we can now consider the chief question of interest that follows the demonstration of the physical existence of the aglomerular nephrons, namely, whether these structures are to be considered only curious residua that mark the wake of the advancing disease or whether they may be objects of functional significance that play some part in the reaction of the organ to its impending destruction.

The morphologic evidence in such a question is of its nature indirect and will be simply stated in summary form. The aglomerular nephron has all the structural requirements for activity that the tubule of the

original nephron possessed. These consist of: continuity of structure, preservation of cytologic detail and adequate supply of blood.[8] Moreover, the nature of its response to the disease processes is similar to that of the tubule of the intact nephron, not only in its development of regressive alterations but in its maintenance of the progressive changes of hypertrophy and hyperplasia, which are generally admitted to be responses to increased functional activity.[9] Whether these progressive changes were acquired before the occurrence of disruption and the formation of the aglomerular tubule or not is inconsequential. A hypertrophied muscle soon loses its bulk if its activity is not continued. The morphologist therefore sees no structural reason to deny the aglomerular nephron functional significance if any is to be granted the tubule of the intact unit. And whatever the uncertainty of the physiologic evidence as to what constitutes tubular activity in the normal kidney, that of the aglomerular nephron must consist of something else than absorptive processes that act on a glomerular filtrate.

In our first investigation of this series we called attention to the structural analogy existing between our model with its large hypertrophic tubule and fibrosed glomerulus and the aglomerular nephron of certain fishes.[1] Marshall[10] and Smith[11] and their co-workers have established the anatomic and functional peculiarities of these organs. The most interesting of these investigations in its relation to our findings is the study of the kidney of the daddy sculpin by Grafflin,[12] for an amazingly close analogy is observed between what appears in the fish to be a normal physiologic involution and the disease process as it occurs in man.

The daddy sculpin begins life with a kidney that contains glomeruli, but with increasing age there occurs a continuous destruction of these elements so that in the fully grown animal the nephrons are aglomerular. The function of the kidney is correspondingly tubular in type, and the

8. A study has not as yet been made of the mitochondrial elements of the aglomerular tubules, but neither is there any adequate description of these cell constituents of the atypical epithelium in the tubules of the intact abnormal nephrons in patients with terminal Bright's disease.

9. The implication of several recent studies of compensatory renal hypertrophy is that the commonly accepted correlation of increased size and increased functional activity may be based rather on theoretical assumption than on actual demonstration. It has been shown by direct comparison of quantitative measurements that these two aspects of compensatory hypertrophy are in remarkably close agreement (Addis, T.; Meyers, B. A., and Oliver, J.: Arch. Int. Med. **34**:243, 1924; Oliver, J.: ibid., p. 258).

10. Marshall, E. K., Jr.: Bull. Johns Hopkins Hosp. **45**:95, 1929.

11. Smith, H. W.: Quart. Rev. Biol. **7**:1, 1932.

12. Grafflin, A. L.: Anat. Rec. **57**:59, 1933; (*a*) p. 68, figure 8, 9; (*b*) p. 69, figure 11.

animal therefore lives by means of its tubules and those auxiliary organs such as gills that are available to water-living forms.

The deletion of the glomeruli in the fish is accomplished by two processes, a degenerative destruction of the tuft and an interruption of the tubule, the tubule itself persisting as an intact continuous structure. If the word "degenerative" is replaced with "inflammatory," the same statement is true of the lesion in the kidney of man in terminal glomerular nephritis. Grafflin's [12] models show two types of tubular interruption: one [12a] in which a glomerulus possesses only a short rudimentary blindly ending tubule that is entirely analogous to our model of stereograms 4 and 6, and another, [12b] interrupted at a considerable distance from the glomerulus, that is similar to our model of stereogram 1.

That the transformation of the human kidney by the disease can never reach the state of completeness that characterizes the involutional process in the fish is readily understandable, since man has none of those auxiliary mechanisms of excretion that are peculiar to water-living forms. He dies, therefore, before his kidney is aglomerular, as perhaps would the fish if it had not gills to help in elimination.

Though in terminal Bright's disease the kidney with its persisting aglomerular nephrons always fails eventually, the question arises as to what part these structures may play in the struggle for survival that occurs as the kidney is slowly destroyed. The mechanism of this survival we have previously considered,[8] and have shown that hyperplasia and hypertrophy may make one persisting structurally intact unit the physical equivalent of twelve normal units. There seems to be no valid morphologic reason why the aglomerular nephrons might not contribute some part at least to the compensatory mechanisms that are sustaining life and why they should not, therefore, be added to the positive side of the balance between reaction and destruction even if their weight is proved in the end to be an inadequate contribution.

It is interesting that the physiologists have for some time been endeavoring to call attention to such possibilities. In a recent summary of the comparative physiology of the kidney, Marshall [13] states: "In the human kidney, where filtration—re-absorption appears under ordinary conditions to play a predominant rôle for the normal urinary constituents, it is possible that under certain pathological conditions the more primitive secretory process in the tubule may be of major importance."

Our description of the structural abnormalities in terminal hemorrhagic Bright's disease seems to make such an assumption not only possible but indeed highly probable.

---

13. Marshall, E. K., Jr.: Physiol. Rev. **14**:154, 1934.

EXPERIMENTAL COLOR CHANGE IN FISH

WILLIAM SAPHIR, M.D.
CHICAGO

In a previous communication [1] experiments were reported concerning the production of the so-called wedding dress or mating coat in the fish Chrosomus erythrogaster. This wedding dress results from a maximal expansion of all the chromatophores of the skin, such as melanophores, xanthophores and erythrophores. As the erythrophores of this fish situated on the abdomen are nearly always in a contracted state (except during the breeding season) the red discoloration of the otherwise white belly is the most striking symptom. In addition, the expansion of the melanophores and xanthophores intensifies the black and yellow colors of back, flanks and root of the fins and thus creates a most beautiful appearance.

For histologic study of the chromatophores the following procedure was found satisfactory: The fish was quickly and painlessly killed and was then fixed as a whole in boiling water, as alcohol or solution of formaldehyde dissolves the pigment. The procedure was carried out as quickly as possible so that a minimum of change in the state of the chromatophores occurred. For purposes of microscopic examination pieces of skin were removed, cleared in glycerin and mounted in balsam (illustration).

The terms "expansion" and "contraction" of chromatophores are used in this study without reference to the actual mode of reaction. The method by which the chromatophores "expand" and "contract" is still an unsettled problem. As the weight of evidence shows that only a movement of the pigment granules takes place, the terms "dispersion" and "concentration" as suggested recently by Parker [2] may be more appropriate. Because of usage, however, the old terms are kept in this paper.

EXPERIMENTAL STUDY

While, as reported previously, a maximal expansion of chromatophores was regularly produced by injection of 1:10,000 solution of yohimbine hydrochloride it was found soon that the same effect was brought about when yohimbine was absorbed through the gills. Fish

---

From the Nelson Morris Institute for Medical Research, Michael Reese Hospital.

1. Saphir, William: Proc. Soc. Exper. Biol. & Med. **31**:864, 1934.
2. Parker, G. H.: Science **79**:428, 1934.

placed in a 1 : 10,000 solution of yohimbine developed a mating coat within from five to fifteen minutes which lasted for twenty-four hours or more, even when the fish were returned to the aquarium immediately after development of the mating coat. This phenomenon showed a marked difference from the moderate and transitory expansion of the chromatophores observed when the fish are kept in the dark, i. e., in a vessel from which light is completely excluded. These fish regain their normal color as soon as they are returned to daylight.

ACTION OF EPINEPHRINE

A simple and reliable method of producing chromatophoric expansion being thus established, it was considered important to study the effect of epinephrine on the expanded chromatophores. Epinephrine has been reported repeatedly [3] to have a marked contracting action on the chromatophores of fish. As, however, for unknown reasons the chromatophores of fish frequently assume spontaneously a stage of partial or complete contraction, the contracting action of epinephrine, in these untreated fish, could not be made sufficiently apparent for use as a biologic test for the presence of epinephrine. When, however, marked expansion of the chromatophores was produced by the injection of yohimbine, 0.1 cc. of epinephrine 1 : 1,000 injected into the fish treated with yohimbine produced a marked contraction of all the chromatophores which was apparent to the naked eye as a generalized transparent paleness and was in striking contrast to the dark and brilliant colors previously produced by yohimbine.

While a generalized pallor was produced with a 1 : 1,000 solution of epinephrine, weaker solutions, from 1 : 10,000 to 1 : 10,000,000, caused a definite local pallor around the site of the injection which was more or less marked according to the strength of the solution. The duration of the chromatophoric contraction lasted according to the strength of the solution from about thirty minutes to several hours but was regularly followed by expansion so that the color caused by yohimbine seemed restored. The impression was gained, therefore, that previously expanded chromatophores, when forcibly contracted by epinephrine, tend to resume their former stage of expansion.

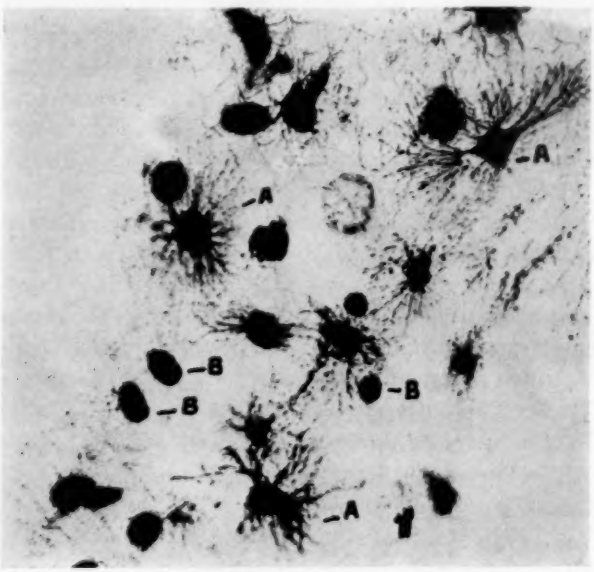
ACTION OF EXTRACT OF THE POSTERIOR LOBE OF THE PITUITARY GLAND

While various hormones and drugs, as reported previously, did not regularly exert definite changes on the chromatophores of the fish, it was found that a characteristic effect was invariably produced by extract of the posterior lobe of the pituitary gland.

---

3. Hewer, H. R.: J. Exper. Biol. **3**:123, 1926.

Preparations from the posterior lobe of the pituitary gland have been reported by some authors [4] to have a contracting effect, and by others,[5] to have an expanding effect on chromatophores of fish. In this species of fish, injection of 0.1 cc. of solution of pituitary U. S. P. regularly produced a marked expansion of the erythrophores associated with a marked simultaneous contraction of melanophores and xanthophores within from ten to thirty minutes. The appearance of the fish to the naked eye, therefore, was that of an intensively red belly and a very pale back and flank. The contraction of melanophores and xanthophores was followed after a few hours by expansion, so that at this time the picture of the full mating coat became again apparent.



Chromatophoric cells of Chrosomus erythrogaster: *A*, expanded; *B*, contracted.

The same effect was obtained when pitressin, the blood pressure-raising fraction, and pitocin, the smooth muscle-contracting fraction, was injected.

These findings seem of interest in view of Zondek and Krohn's [5] work on intermedin (the chromatophore principle of the intermediate lobe of the pituitary gland). These authors found that in Phoxinus levis isolated expansion of erythrophores was characteristic of the action of their pituitary pigment hormone intermedin. In Chrosomus erythrogaster, a near relative of Phoxinus levis, isolated expansion of erythro-

4. Odiorne, J. M.: Proc. Nat. Acad. Sc. **19**:745, 1933.

5. Zondek, B., and Krohn, H.: Klin. Wchnschr. **11**:405, 849 and 1293, 1932.

phores can be produced with preparations from the posterior lobe of the pituitary gland. The assumption that a specific pituitary pigment hormone exists does not, therefore, seem warranted.

SUMMARY

Complete expansion of chromatophores in the fish Chrosomus erythrogaster is brought about by a 1 : 10,000 solution of yohimbine hydrochloride either administered subcutaneously or absorbed through the gills.

Complete contraction of expanded chromatophores is produced by epinephrine.

Preparations from the posterior lobe of the pituitary gland exert an isolated expansion of the erythrophores and simultaneous contraction of the melanophores and xanthophores.

COMPENSATORY HYPERTROPHY OF THE REMAINING KIDNEY AFTER NEPHRECTOMY FOLLOWING TRANSPLANTATION OF ITS URETER INTO THE DUODENUM

JESSE L. BOLLMAN, M.D.
AND
FRANK C. MANN, M.D.
ROCHESTER, MINN.

During the course of an investigation on the effects of bilateral ureteroduodenostomy and ureterojejunostomy we observed few changes in the kidneys which we ascribed to ascending infection or to hydronephrosis. Some of the animals survived from fifteen to twenty days and gave evidence of tremendous accumulations of urea in the blood, while the concentration of certain other urinary constituents, such as creatinine and uric acid, did not change until extremely late. In repeating some of this work, we found that the time of survival was reduced by unilateral nephrectomy at the time of ureteroduodenostomy and that the chemical changes in the blood were essentially similar to those that follow bilateral ureteroduodenostomy, except that the value for blood urea did not reach such high levels and that the concentration of creatinine in the blood increased more rapidly. In these experiments we were able to compare the histologic appearance of the normal kidney removed at operation with that of the remaining kidney and were also able to rule out almost completely changes produced by infection or hydronephrosis. The most striking observation was that the remaining kidney rapidly increased in size.

The activating causes of renal hypertrophy have been investigated by the addition of various substances to the diet. Newburgh and Johnston;[1] Mackay, Mackay and Addis,[2] and Wilson[3] have expressed essential agreement that diets rich in protein stimulate hypertrophy of the kidney. There also appears to be greater stimulation from certain proteins, although the various amino-acids tried were apparently nonspecific and gave only the response indicated by their nitrogen content. Wilson[3] has expressed the belief that some intermediary of protein metabolism is largely responsible for hypertrophy of the kidneys because of the greater response obtained from proteins than from urea

From the Division of Experimental Medicine, the Mayo Clinic.

1. Newburgh, L. H., and Johnston, M. W.: J. Clin. Investigation **10**:153, 1931.

2. Mackay, L. L.; Mackay, E. M., and Addis, T.: J. Nutrition **4**:379, 1931.

3. Wilson, H. E. C.: Biochem. J. **27**:1348, 1933.

or salts. Hartman [4] studied five methods of increasing the urinary constituents in the body by slow, continued reabsorption of urine. By providing urinary fistulas into the intestinal tract he found that degenerative changes were produced in the kidneys by an increase in the urinary constituents in the body. Hypertrophy of the kidneys accompanied or preceded these degenerative changes. Similar changes were produced in the kidneys of animals which received concentrated urine, synthetic mixtures of urea and urinary salts or even urea or mixtures of salts alone, administered more or less continuously by intestinal fistulas. Allen [5] has found that hypertrophy of the remaining kidney following unilateral nephrectomy is increased by increased physiologic work for the kidney, whether this be due to increased intake of protein, urea or sodium chloride in the diet.

METHODS

Adult dogs were used in these experiments. Laparotomy was performed under ether anesthesia, using aseptic technic. Both kidneys were measured in situ with the aid of micrometer calipers. In this series measurements of both kidneys were found to be identical. In most animals the right kidney was removed and the ureter of the left kidney was transplanted into the duodenum or upper part of the jejunum by the technic described by Beaver and Mann.[6] Immediately following operation the animals were placed in large metabolism cages so that any excreta or vomitus could be collected. Food and water were given during the course of the experiment, but for the most part food was taken sparingly, so that the changes observed were essentially similar to those of fasting animals under similar conditions. The animals were killed after the number of days indicated; most of the animals were in fair general condition at this time. At necropsy the kidney was again measured and weighed, and microscopic sections were made to compare with the sections from the normal kidney that previously had been removed. Results of some experiments were discarded because of obvious infection of the kidney and only those were retained in which there was no gross or microscopic evidence of infection.

Control experiments for the operative procedure consisted of similar transplantation of the ureter of one kidney into the duodenum without disturbing the other kidney except to take measurements. In these experiments there was but little evidence of retention of urinary constituents in the blood, and very little difference was found in the weight of the kidneys at necropsy.

Removal of one kidney at operation and transplantation of the ureter of the other into the sigmoid flexure were followed by somewhat greater retention of urea in the blood, but in no case was the retention comparable to that obtained by transplantation of the ureter to the upper part of the small intestine. The kidney of which the ureter was made to empty into the sigmoid flexure gave some evidence of hypertrophy, but this did not exceed 15 per cent in the first ten days following operation.

---

4. Hartman, F. W.: J. Exper. Med. **58**:649, 1933.
5. Allen, R. B.: Unpublished data.
6. Beaver, M. G., and Mann, F. C.: Ann. Surg. **95**:620, 1932.

RESULTS

After the second day following transplantation of the ureter of one kidney into the duodenum or upper part of the jejunum and the removal of the other kidney, the remaining kidney appeared definitely enlarged

TABLE 1.—*Renal Hypertrophy Following Unilateral Nephrectomy and Transplantation of the Other Ureter into the Small Intestine*

Dog	Days After Operation	Increase in Blood Urea Nitrogen, Mg. per 100 Cc.	Increase in Blood Creatinine, Mg. per 100 Cc.	Weight of Kidney Removed, Gm.	Weight of Kidney Remaining, Gm.	Increase in Weight of Remaining Kidney, per Cent
1	3	75.2	3.65	45.5	50.0	10
2	5	196.0	12.70	35.0	58.0	65
3	5	221.4	7.85	30.0	61.0	103
4	6	278.6	10.70	29.5	56.0	90
5	8	342.0	9.77	32.0	51.0	60
6	9	232.5	5.00	19.0	32.0	68
7	9	251.7	5.13	49.0	85.0	73

TABLE 2.—*Blood Chemistry Following Transplantation of the Left Ureter into the Jejunum and Right Nephrectomy*

Days After Operation	Urea Nitrogen, Mg. per 100 Cc.	Creatinine, Mg. per 100 Cc.	Chlorides, Mg. per 100 Cc.	Phosphates, Mg. per 100 Cc.
0	21.8	2.14	356	3.79
1	49.6	2.97	316	5.40
2	112.2	3.02	330	6.35
3	116.0	2.97	368	8.63
4	135.5	2.84	405	7.23
5	112.3	2.00	420	4.67
6	144.8	3.47	375	3.20
7	200.1	3.94	344	7.50
8	251.7	5.13	362	8.10

TABLE 3.—*Blood Chemistry Following Transplantation of Right Ureter into Duodenum, Left Kidney and Ureter Being Undisturbed* *

Days After Operation	Urea Nitrogen, Mg. per 100 Cc.	Creatinine, Mg. per 100 Cc.	Chlorides, Mg. per 100 Cc.	Phosphates, Mg. per 100 Cc.
0	14.7	2.10	390	3.03
1	19.7	2.38	280	3.64
2	28.5	2.14	365	3.84
3	19.7	2.23	370	3.72
4	12.5	2.22	390	3.36
5	8.0	2.14	385	3.66
6	7.0	2.57	380	3.80

* After six days the right kidney weighed 49.0 Gm.; the left kidney, 47.2 Gm.

(table 1, fig. 1). The capsular veins appeared dilated and the pelvis of the kidney and the ureter were only slightly dilated, except in a few instances in which infection had occurred in which case the results were not included in this study. In color and consistency the kidneys were essentially normal. The weight after the fifth day was from 60 to 103 per cent greater than that of the opposite kidney which had been removed

at operation, and the measurements of the remaining kidney were also found to be increased in all dimensions.

Microscopic sections of the remaining kidneys appeared quite uniform. The glomeruli were about a third larger than normal in diam-

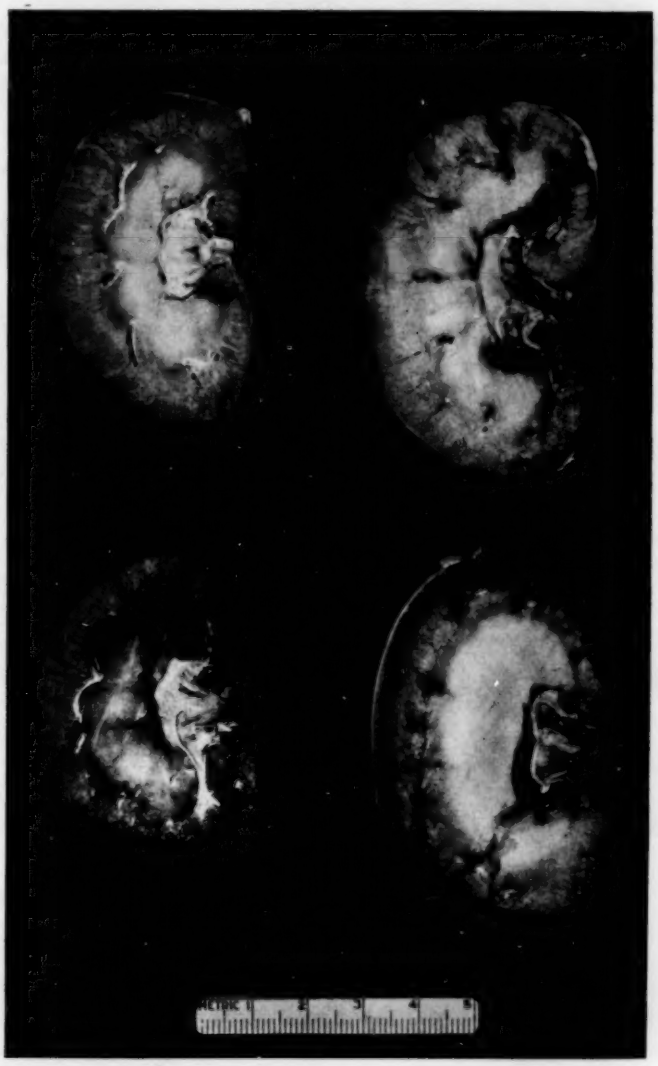


Fig. 1.—Hypertrophy of the kidney following removal of the left kidney and transplantation of the right ureter into the duodenum. On the left are the normal kidneys removed at operation from dogs 2 and 4, on the right the kidneys removed five days (upper) and six days (lower) after ureteroduodenostomy.

eter, and most of this increase appeared to be due to increase in the size and the apparent number of capillary tufts of the glomeruli. Bow-

man's capsule appeared dilated, but the fluid content of the glomeruli did not seem to be materially increased. An occasional mitotic figure (fig. 2) was found in the capsular epithelium, but none was observed in the glomeruli. The cells of the tubules appeared swollen, but the lumens of the tubules were not enlarged. Many mitotic figures were present in the tubular epithelium, and the cells appeared irregular in outline and granular, to an extent which would be consistent with early degenerative changes. The collecting tubules, the pelvis of the kidney and the ureter appeared essentially normal. All the kidneys in a group of control animals appeared normal.

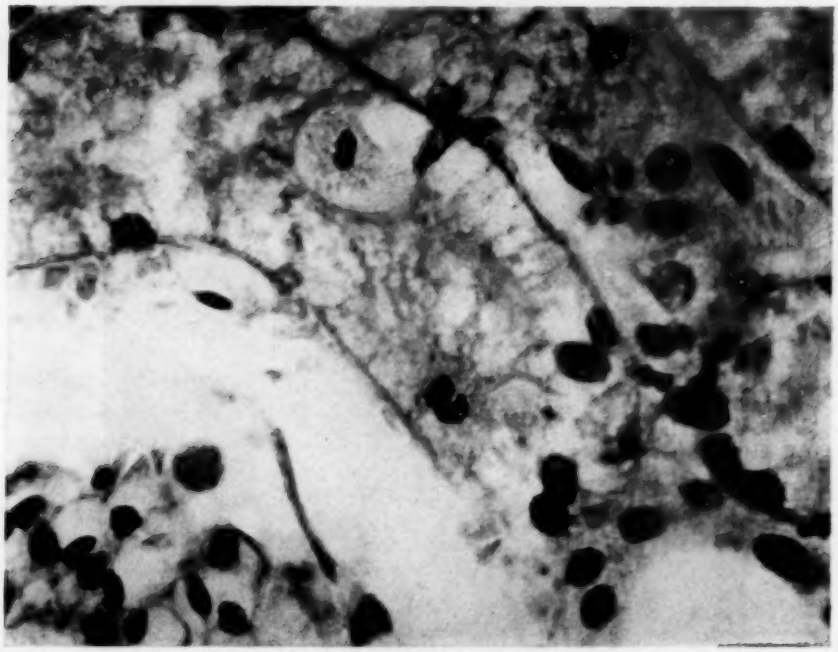


Fig. 2.—Mitotic figures and granular degeneration in the cells of the tubular epithelium of the kidney five days after unilateral nephrectomy and ureteroduodenostomy.

The changes that occurred in the chemical constituents of the blood were similar to those found following bilateral nephrectomy, except that the concentration of creatinine in the blood did not increase for the first few days and did not rise to as high a percentage (tables 2 and 3). The concentration of nonprotein nitrogen in the blood increased rapidly; much of the increase was due to the increase in the urea of the blood, although considerable increase in the undetermined nitrogen occurred. The feces contained a large amount of nitrogenous substances which varied greatly in amount; from 0.25 to 4 Gm. of nitrogen

was excreted from the body daily in this way. The rise in the concentration of urea in the blood was not always progressive; occasional decreases were observed which were due to excessive loss of urea in the feces and vomitus. The uric acid content of the blood did not change, and the allantoin content was not determined. The content of phosphates and sulphates in the blood gave evidence of definite, although irregular, increases, whereas the chloride content of the blood was unchanged.

COMMENT

From these results it would appear that retention of urinary products in the blood hastened and increased the hypertrophy which occurred following unilateral nephrectomy. While no specific substance could be demonstrated in experiments of this type it is interesting to note that the blood urea and the phosphates and sulphates of the blood were markedly increased, whereas the concentration of chlorides remained unchanged. Hartman [4] found greater changes after administration of urea and mixtures of inorganic salts than after administration either of urea or of salts alone. We are inclined to look on the increase found in the creatinine content of the blood as indicative of renal impairment rather than of simple retention due to continued absorption of this substance from the intestine. In our earlier studies [7] on transplantation of both ureters into the duodenum, creatinine increased in the blood only after eight or ten days, when some injury to the kidney could be demonstrated; the blood creatinine increased in the first two or three days in that series and also in the animals of this series in which infection was present. The increase in creatinine of the blood appears much earlier if one kidney is removed and one ureter transplanted to the duodenum than it does if both ureters are transplanted to the duodenum. There is also a greater change in the histologic characteristics of the one kidney than when both are present, so that it would appear that two kidneys are better able to withstand the accumulation of urinary products than is a solitary kidney.

SUMMARY

Hypertrophy of the remaining kidney after nephrectomy is greatly increased by accumulation of urinary products in the blood following transplantation of the ureter so that the urine drains into the duodenum. The kidney increases in weight from 60 to 100 per cent within nine days. The glomerular tufts are enlarged, but most of the increase appears to be due to swelling and multiplication of the cells of the tubules in which there is some evidence of early degeneration. Creatinine apparently does not accumulate in the blood under these conditions until degeneration of the kidneys appears.

---

7. Bollman, J. L., and Mann, F. C.: Proc. Soc. Exper. Biol. & Med. **24**:923, 1927.

LIGATION OF THE COMMON BILE DUCT IN THE CAT

HAROLD L. STEWART, M.D.
AND
MARSHALL M. LIEBER, M.D.
PHILADELPHIA

The cat appears to have been the first animal in which obstructive jaundice was produced experimentally for the purpose of investigating phenomena associated with biliary stasis. Malpighi,[1] in 1687, tied the cystic and common bile ducts in cats and disproved the contention of Fallopius [2] that bile flows from the gallbladder to the liver; the results of these experiments and those of a similar nature performed by Pechlin [3] were published by Hoffmann [4] in 1703. This procedure was also employed by Brodie [5] and others interested chiefly in the physiologic and biochemical aspects of digestion and biliary secretion. These observers noted the occasional reestablishment of biliary circulation a week or so after complete obstruction, but reported few other observations of pathologic interest. Mayer,[6] who is credited with being perhaps the first to study particularly the hepatic lesions dependent on total biliary stasis, noted dilatation of bile ducts, fatty changes in the liver and increase of portal connective tissue in cats dying within twelve days, usually of peritonitis. Legg [7] ligated the common bile duct in sixteen cats, twelve of which survived a sufficient length of time for significant lesions to appear. Clinically the animals became markedly jaundiced and emaciated; at autopsy large, firm livers with parenchymal atrophy were noted and, what is more important, "an increase in connective tissue which can be made out within a few hours and which

From the Pathological Laboratories of the Jefferson Medical College and Hospital, and the Jefferson Hospital Tumor Clinic.

1. Malpighi, M.: De hepate, in Opera omnia, Lugd. Bat., P. Vander Aa, 1687, vol. 2, chap. 7, p. 264.

2. Fallopius, G.: Observationes anatomicae, in Vesalius, A.: Opera omnia anatomica et chirurgica, cura, Hermanni Boerhaave et Bernhardi Siegfried Albini, Lugd. Bat., J. du Vivie et J. et H. Verbeck, 1725, vol. 2, p. 811.

3. Pechlin, quoted by Hoffmann.[4]

4. Hoffmann, J. M.: Idea machinae humanae anatomico-physiologica, Altdorfi, H. Meyer, 1703; quoted by Cameron, G. R., and Oakley, C. L.: J. Path. & Bact. **35**:769, 1932.

5. Brodie, B.: Quart. J. Sc., Literature & Arts **14**:341, 1823.

6. Mayer: Med. Jahrb., Wien **2**:133, 1872.

7. Legg, J. W.: St. Barth. Hosp. Rep. **9**:161, 1873.

progressively increases destroying the tissue of the liver before it." Neither the aforementioned investigators nor Foà and Salvioli,[8] who established the significant fact that various experimental animals react differently to ligation of the common bile duct, maintained strict asepsis in their operative procedures, and their results were further complicated in many instances by the use of chloroform, a hepatotoxic agent, as an anesthetic. In cats surviving many months after ligation of the left branch of the common bile duct, Harley and Barratt[9] noted considerable atrophy of the hepatic lobules in the affected lobes, fibrosis with small collections of leukocytes and dilatation of the larger bile ducts with hyperplasia and varicosities of the small radicles.

Although the cat is particularly suitable for this type of investigation, apparently no serious attempt has been made in recent years to utilize this animal in the study of hepatic lesions associated with biliary stasis. In view of the many recent advances in the understanding of several aspects of the physiologic and pathologic processes of the liver, such a study appears to be of interest and significance. The present investigation of the changes in the liver and bile passages following ligation of the common bile duct in the cat was instituted for the purpose of establishing a pathologic standard with which might be compared the effects of a variety of other related procedures at present under investigation.

MATERIALS AND METHODS

The following observations are based on a study of seventy-five cats maintained under identical conditions. Of these, ten from the same litters as the animals subjected to operation were kept as controls. The remainder were anesthetized with ether, and the abdomen clipped and washed with Harrington's solution, followed by alcohol. A midline incision was made from the xiphisternum downward for a distance of 5 cm. The edges of the liver were elevated, the duodenum pulled downward and a double ligature placed on the isolated common bile duct which was then severed; in alternate animals the cystic duct was doubly ligated and severed, and the gallbladder extirpated. A biopsy of the liver was made in many instances. As a result of stitch abscess, local or generalized peritonitis or postmortem changes, nineteen cats were discarded, leaving forty-six cats in which the surgical incisions healed by first intention. At intervals of from one hour to forty-two days, the cats either died or were lightly anesthetized with ether and bled from the heart. Pieces of liver were fixed in a solution of formaldehyde, U. S. P. (1:10), Zenker's fluid or absolute alcohol; some were frozen and sectioned and stained for fat and glycogen; the rest were embedded in paraffin and cut and stained by the following methods: the phosphotungstic acid and hematoxylin stain; the hematoxylin and eosin stain; methylthionine chloride, U. S. P. (methylene blue); Van Gieson's and Mallory's connective tissue, and Verhoeff's elastic tissue stains; the ferric ammonium sulphate and hematoxylin stain; Mallory's potassium ferrocyanide stain, and the oxidase reaction.

8. Foà, P., and Salvioli, G.: Arch. per le sc. med. **2**:1, 1877.

9. Harley, V., and Barratt, W.: Brit. M. J. **2**:1743, 1898.

GROSS CHANGES

Proximal to the ligature, there was a progressive but irregular dilatation of all the biliary passages, which was roughly proportional to their previous size and the amount of hepatic tissue with which they were continuous. With the gallbladder removed, the common duct above the ligature frequently formed a large sacculated cyst, measuring as much as 4 by 4 by 3 cm. The walls of the gallbladder and larger bile ducts became attenuated and subsequently underwent a slight thickening, their contents rapidly becoming translucent, white, viscid and ropy in several cases. Strawberry gallbladders were noted in two instances. In the early stages, the liver was usually enlarged, smooth, and a mottled yellow, yellowish brown or olive-drab which turned green on exposure to air. Later on, it became finely granular; varicose ducts appeared on the surface, and the organ felt tough, firm and somewhat cystic, cutting with increased resistance and collapsing when the ducts were opened. The cut surface disclosed accentuated lobular markings, dilated biliary passages, visible small islands of periportal connective tissue and occasionally small yellowish-gray punctate foci of necrosis. In two cats, dark red, sharply demarcated, triangular areas of infarction were noted in the lower borders of several lobes.

MICROSCOPIC CHANGES

Pigmentation.—The degree of pigmentation, which was comparatively inconspicuous, did not always vary directly with the duration of stasis or with the clinical manifestations of jaundice. It was usually most marked in the inner third of the lobule which was, however, sometimes involved peripherally in focal areas or diffusely. It occurred as indistinct clusters of greenish-yellow or golden granules, concentrated especially about the circumferences of the vacuoles and along the borders of the canaliculi in that part of the cytoplasm of the hepatic cells remote from the nucleus, which might appear surrounded as by a halo. Pigment was present in the epithelial cells of the bile ducts, either free or in phagocytic cells in the sinusoids and the perivascular tissue spaces, about the areas of necrosis, and in the form of small oval collections, lying within and against the thickened portal radicles. It was also to be seen as diffuse staining material in the perivascular tissue spaces and dilated canaliculi or, in the late stages, as biliary thrombi or masses in Hering's canals, Letulle's collecting tubules and other canaliculi at the peripheries of the lobules.

Regressive Changes.—These might be sporadic or diffuse, but were regularly present in the inner portion of the lobule. During the first five days, they consisted of varying degrees of vacuolation, degeneration and necrosis of the hepatic cells, the parenchyma being restored repeat-

edly to a fairly normal condition by both amitotic and mitotic division. The tissue immediately beneath the capsule of the liver presented a narrow pale-staining zone of swollen, finely reticulated hepatic cells with sharply outlined borders and indistinct oval nuclei which frequently appeared as basophilic smudges. The degenerative lesions remained stationary in this area unless complete necrosis supervened. In the later stages there was less and less tendency for regeneration to occur, although the regressive changes progressed the hepatic cells

Fig. 1.—A focal midzonal necrosis lying adjacent to a large branch of the hepatic vein, the wall of which over the area of involvement is necrotic and covered on its inner aspect by a thrombus composed chiefly of disintegrated platelets and a few white cells; × 150.

being compressed into parallel rows about the dilating bile ducts, with single cords presenting a lumpy, irregular, thinned-out appearance.

Focal Necroses.—The initial changes eventuating in this lesion, which appeared in typical form at the end of five hours, were difficult to ascertain but were thought to be represented by coagulation necrosis with accentuation of cellular outlines and lumpiness of the cytoplasm involving groups of from ten to fifteen cells within the first two hours of stasis. When definitely recognizable the lesions were oval or tri-

angular and tended to be situated in the midzonal portions of the lobules but might enlarge in the direction of the central vein and portal radicle, with which they tended to maintain a rather constant relationship. In several instances the bases of these lesions extended to involve branches of the portal and hepatic veins and produce necrosis of the corresponding portion of the vessel wall with the resulting formation of a platelet thrombus (fig. 1). The focal necroses were sharply demarcated peripherally by compressed, viable parenchyma infiltrated with many macrophages. Within the lesion, the nuclei of the hepatic cells became swollen and acidophilic, and faded out entirely. A portion of the cytoplasm became finely reticulated, and a hyaline change occurred along the borders of both the perisinusoidal tissue spaces and the canaliculi, which ultimately faded and disappeared into pale, irregular lines. Total autolysis then supervened, leaving only remnants of a swollen reticulated network, infiltrated with macrophages and polymorphonuclear leukocytes and impregnated with granules of glycogen and small lipoid droplets, which were probably derived from the necrotic hepatic cells as a result of the phanerosis of fat. The sinusoids were usually not involved in the early stages but later might show accentuation of their reticular walls, rupture, hyaline thrombi and an increase in the content of the phagocytic cells which invaded and digested the necrotic hepatic cells. The phagocytes loaded with engulfed material escaped by way of the sinusoids and central vein and along the perivascular tissue spaces to the portal radicles, where they were often held up for a short time prior to entering the lymphatic vessels. Coincident with the removal of the necrotic material and other débris, hepatic cells in the form of short irregular cords, the tips of which sometimes underwent secondary degeneration and necrosis, grew in from all parts of the periphery to replace the lost parenchyma. Regeneration was most active on the side of the lobule next the portal radicle and was characterized chiefly by the appearance of binucleated and multinucleated hepatic cells with hypertrophied and hyperchromatic nuclei rarely seen in mitosis. In one of the cats, within several of the focal areas of necrosis, a marked deposition of fibrin occurred which served as a framework for the proliferation of connective tissue and the subsequent retraction of the hepatic cords in this vicinity in the direction of the reparative process.

Hyaline Necroses.—Within the first twenty hours, sporadically throughout the lobules but especially just beneath the capsule of the liver, the hepatic cells underwent hyaline necrosis and formed uniformly narrow zones or large wedge-shaped, sharply demarcated areas, the apexes of which occasionally maintained a relationship with the portal radicles. The peritoneum overlying these lesions was frequently cov-

ered by a fibrinohemorrhagic exudate. The regressive changes consisted of an accentuation of the cytoplasmic reticulation which is regularly present in the subcapsular parenchyma and an entire fading out of the smudge of chromatin material representing the nucleus. The cytoplasm in the majority of the hepatic cords in the area was converted into hyalinized, conglutinated, lumpy, acidophilic masses either disintegrating spontaneously or being phagocytosed by macrophages and polymorphonuclear leukocytes, which after invading the cell migrated to the spot from which the nucleus faded. The sinusoids in the area were compressed and often thrombosed. The lesion persisted in this form for only a short time when the viable hepatic cells in the adjoining parenchyma, without showing binucleation or mitotic figures to an appreciable extent, appeared to move in a sheet toward the capsule, against which were compressed the phagocytes and necrotic material, which was rapidly being evacuated. The peritoneal exudate underwent resolution and organization, leaving only a residue of slight capsular thickening.

After the fifth day, but more particularly toward the termination of biliary stasis, an acidophilic, hyaline type of necrosis made its appearance involving sporadically groups of from six to eight cells and smaller or larger portions of single or several lobules in the vicinity of the portal radicles. The necrotic area was invaded by phagocytic cells and organizing fibrous tissue, carrying a few newly formed capillaries which regressed with the complete fibrosis of the lesion. The newly formed connective tissue, following its usual course of slow contraction, pinched off and destroyed the vascular and biliary channels of the portal radicles in this vicinity. Newly formed necrotic lesions were superimposed on old ones (fig. 2), resulting in an extreme degree of concentric avascular fibrosis of the portal radicles and their included structures. Small amounts of bile pigment were deposited in focal areas about the periphery of, or in small clefts within, these fibrotic nodules shortly after their development.

Bile Duct Changes.—The mucosa of the larger ducts underwent enormous proliferation, particularly after the tenth day, when it presented a papillary and adenomatous arrangement composed of multiplying cylindric epithelial cells several layers thick, supported by hyperemic fibrous tissue with a few inflammatory cells. The smaller ducts showed mitotic figures and budding within the first twenty-four hours and grew out from the portal radicles in the form of cords of cells supported by connective tissue, but with no demonstrable lumens. They insinuated themselves between the hepatic cells of adjacent lobules, the majority of which, after the twenty-first day, were surrounded by a collar of these proliferating elements. This resulted in irregularity and atrophy of the lobules, retraction of the capsule of the liver and

pitting of the surface in localized areas. The destruction of newly proliferated bile ducts was effected in either of two ways. In some a pink hyaline secretion accumulated, which distended their lumens and caused compression and necrosis of the epithelial cells lining them. These liberated a stringy basophilic substance which was ultimately removed by the action of phagocytic cells. In the later stages, many of the bile ducts, both large and small, succumbed to destructive changes attending the development and subsequent organization of the hyaline necroses (fig. 2).

Fig. 2.—Fresh areas of hyaline necrosis superimposed on older ones which have become organized. Bile ducts and vascular structures may be seen in various stages of destruction. A narrow rim of viable hepatic cells is present along the left upper quadrant; × 100.

Vascular Changes.—In the early stages there was usually dilatation of the branches of the hepatic and portal veins and of the lymphatic vessels in the portal radicles. The sinusoids were usually compressed by dilating bile ducts at the periphery of the lobule and often showed focal or diffuse hyperemia in other areas and, later, hyaline and fibrinous thrombi which were frequently unassociated with areas of focal midzonal necrosis. There was a constant increase in the reticulum and connective tissue about all the vascular structures, particularly in the

portal radicles, in which eventual obliteration occurred as a result of the organization of the hyaline necroses.

Areas of infarction with a complete necrosis of the greater portions of several lobes, due to thrombosis of large branches of the hepatic artery and portal vein, were noted in two cats killed on the fifteenth day. The intrahepatic line of demarcation between the infarct and the remaining parenchyma was sharp, but irregular, because of the maintenance of circulation around the portal radicles and sublobular veins in the uninvolved tissue. A narrow zone of viable hepatic parenchyma, separated by hyperemic sinusoids, was constantly maintained immediately beneath the capsule of the liver. The outer margin of the necrotic lesion was invaded to only a slight extent by macrophages and polymorphonuclear leukocytes, which passed out of the hyperemic vessels in this region. The structural arrangement of the necrotic tissue within the area of infarction was well maintained with a tendency for the fibroblasts, endothelium and epithelium of the bile ducts, in this order, to resist longest the effects of the regressive change. The necrotic hepatic cells formed hyaline, conglutinated masses, except in the inner portion of the lobule, where autolytic changes were present leaving a thickened, irregular, beaded reticulum. In some places the cytoplasm of the hepatic cells was pale and only moderately acidophilic, with the shadowy outlines of nuclear structures still evident. The perivascular tissue spaces were prominent and the lumens of the sinusoids diminished in size, and either empty or filled with granular débris and the shadowy outlines of a few erythrocytes.

Regeneration.—Regenerative processes, characterized by budding and fission of the nuclei which led to binucleation and multinucleation and the formation of hypertrophic and hyperchromatic hepatic cells, were noted within eight hours to be replacing the necrotic areas of the parenchyma. By the end of forty-eight hours, this type of regeneration gave way to the mitotic division of hepatic cells which predominated from that time onward, although both processes contributed to the maintenance of the normal hepatic parenchyma, which was subject, however, to repeated injuries throughout the entire course of stasis. Regeneration lagged after the fifteenth day, as evidenced by the more frequent occurrence of regressive lesions which tended to progress, and only the occasional appearance of mitotic figures and binucleated cells.

COMMENT

The etiology and pathogenesis of the various types of regressive change in the hepatic parenchyma and biliary passages associated with experimental and clinical obstructive jaundice have been fully considered

in the papers of Rous and Larimore,[10] McMaster, Broun and Rous,[11] Judd, Counseller and McIndoe,[12] MacMahon, Lawrence and Maddock,[13] MacMahon and Mallory,[14] Rabl,[15] Cameron and Oakley,[4] Lieber and Stewart[16] and Stewart and Lieber.[17] The present discussion will be restricted, therefore, to those phases of the problem which are of particular interest from the standpoint of our observations.

The sporadic degenerative changes and retardation of regenerative processes in the livers of cats in the late stages of biliary stasis may be due chiefly to obliteration of the larger vascular structures incident to the repair of the hyaline necroses. In the early stages, however, the fact that sporadic regressive changes are extremely inconstant supports the belief that individual susceptibility and extraneous factors, toxic, dietary or infectious, may play an important rôle in their production. Accidental infection at the time of operation vitiated the earliest studies of these regressive lesions but, beginning with Belousssow's[18] work in 1881, much more attention was directed toward maintenance of asepsis, so that the possible influence of this factor has been omitted from consideration by most subsequent observers. However, infection, perhaps spontaneous, in the form of transient bacteremia, may be of importance, especially since the protective functions of the reticulo-endothelial cells possibly are impaired by their saturation with bile pigment. Furthermore, the liver lies directly in the pathway of the portal blood, which may carry bacteria from the gastrointestinal canal. Magner and Hutcheson,[19] Narita[20] and Fiessinger and Cottan[21] studied the changes which occurred in the liver and biliary passages following the injection of a variety of micro-organisms into the ear vein, portal vein and hepatic artery of animals. Regressive hepatic parenchymal lesions occurred promptly, being most marked at

10. Rous, P., and Larimore, L. D.: J. Exper. Med. **31**:609, 1920; **32**:249, 1920.

11. McMaster, P. D.; Broun, G. O., and Rous, P.: J. Exper. Med. **37**:685, 1923. Rous, P.: Am. J. M. Sc. **170**:625, 1925.

12. Judd, E. S., and Counseller, V. S.: (*a*) J. A. M. A. **89**:1751, 1927. (*b*) Judd, E. S., and McIndoe, A. H.: The Collected Papers of the Mayo Clinic, Philadelphia, W. B. Saunders Company, 1929, vol. 21, p. 158. (*c*) Counseller, V. S.: Ann. Surg. **87**:210, 1928.

13. MacMahon, H. E.; Lawrence, J. S., and Maddock, S. J.: Am. J. Path. **5**:631, 1929.

14. MacMahon, H. E., and Mallory, F. B.: Am. J. Path. **5**:645, 1929.

15. Rabl, R.: Beitr. z. path. Anat. u. z. allg. Path. **86**:135, 1931.

16. Lieber, M. M., and Stewart, H. L.: Arch. Path. **17**:362, 1934.

17. Stewart, H. L., and Lieber, M. M.: Arch. Path. **18**:30, 1934.

18. Beloussow, P. N.: Arch. f. exper. Path. u. Pharmakol. **14**:200, 1881.

19. Magner, W., and Hutcheson, J. M.: Canad. M. A. J. **27**:469, 1932.

20. Narita, T.: Ztschr. f. d. ges. exper. Med. **80**:303, 1932.

21. Fiessinger, N., and Cottan, R.: Presse méd. **50**:1009, 1933.

the end of twenty-four hours and tending to undergo resolution by the fifteenth day, after which time active immunity was established. Their studies confirmed the importance of the liver as a mechanism of defense against bacterial invasion, the ability of the animals to withstand infection being diminished to a considerable degree by preliminary ligation of the bile ducts and reticulo-endothelial blockade.

Focal Midzonal Necroses.—The erroneous statement that focal midzonal areas of necrosis do not occur in cats subjected to ligation of the common bile duct has been perpetuated throughout medical literature. These lesions appear in typical form within five hours, are most numerous between the twenty-fourth and forty-eighth hours, are present in many cases during the first eight days and then decrease steadily, being entirely absent after the thirteenth day. No plausible explanation is to be offered for the fact that, even when exceedingly numerous, they may be limited to a small portion of the liver, although this distribution may be accounted for on the basis of stream lines in the portal vein. The hypothesis that these lesions, which have been compared morphologically to infarcts, are dependent on some vascular disturbance is not substantiated by our observations. The majority of the sinusoids within the lesion are usually open to the flow of blood, at least in the early stages. When sinusoidal thrombosis does occur, the mechanism of its production is probably similar to that involved in the pathogenesis of platelet thrombi overlying necrotic portions of the walls of large venous branches in the vicinity of these focal midzonal areas of necrosis. These are secondary manifestations, probably dependent on the elaboration of toxic split-protein products, which, by causing injury to the reticular walls of the sinusoid, may result in hemorrhage and the deposition of fibrin. This reasoning is further substantiated by the fact that focal midzonal areas of necrosis are absent in many large localized areas of sinusoidal thrombosis. In the cat, the incidence of focal midzonal areas of necrosis is in inverse ratio to the degree of vascular constriction by the dilating bile ducts; indeed, the areas of necrosis can no longer be found at a time when the constriction is most marked. Furthermore, the finely adjusted compensatory mechanisms for overcoming resistance to the flow of blood, the numerous anastomoses between the blood vessels entering the liver by various avenues and the nature and efficiency of the collateral circulation in the cat argue against the vascular origin of these lesions.

No evidence is to be adduced from our experiments for or against the theory that these necroses are due to the toxic effects of bile diffused or extruded from rupture of the smaller biliary passages. It has been stated that in the rabbit and guinea-pig, both of which secrete relatively large amounts of bile at comparatively low pressures, these lesions appear

in the liver shortly after the common duct is obstructed; on the other hand, in the dog and cat in which the amount of bile secreted is small but the average pressure of the bile high, necroses do not appear, the inference being that the total quantity of bile produced is of more importance in this connection than the pressure at which it is secreted. This theory is untenable for, as we have noted, in the cat focal necroses do develop despite the fact that it secretes the smallest relative amount of bile per day (Quincke and Hoppe-Seyler [22]) at the highest average pressure (Herring and Simpson [23]).

On the basis of our previous observations on human material with complete and permanent stasis, we were inclined to think that the focal necroses do not progress, regenerate or heal until surgical decompression is instituted, after which they are rapidly effaced by regenerating hepatic cells. Many investigators suspected, however, that the necrotic areas underwent regeneration in other species of animals during the course of permanent biliary stasis, since they had largely disappeared from sections obtained in the late stages of this condition. We have been able to follow objectively the exact manner in which these lesions are ultimately disposed of in the cat. The parenchyma in the area of necrosis is restored to normal by regeneration of hepatic cells and by the ingrowth of cords of liver cells from the uninvolved tissue at the periphery. Organization occurred in one instance in which there had been a preliminary exudation of fibrin into the necrotic areas.

Hyaline Necroses.—The hyaline necroses which occur in the early stages of biliary stasis in cats differ from those present in the late stages in every essential respect other than appearance. In the late stages they are situated periportally, undergo organization and contribute considerably to the increase of connective tissue. Those occurring early, on the other hand, are present chiefly beneath the capsule of the liver, tend to spare the tissue about the portal radicles and undergo resolution with restoration of the area of involvement to a comparatively normal condition. No plausible explanation is apparent for this difference in distribution and ultimate disposition, although in a general way it may be stated that regeneration is extremely active in the early stages, whereas subsequently this process is retarded and supplanted by active proliferation of connective tissue. It is difficult to believe that the subcapsular areas of necrosis are due to vascular disturbances, since the blood supply of the hepatic cells in this area is derived not only from the branches of the hepatic artery but also from collateral capsular channels, which adequately maintain the nutrition of the underlying

22. Quincke, H., and Hoppe-Seyler, G., in Nothnagel, H.: Specielle Pathologie und Therapie, Vienna, A. Hölder, 1899, vol. 18, p. 59.

23. Herring, P., and Simpson, T.: Proc. Roy. Soc., London, s. B **79**:517, 1907.

tissue even when infarction occurs. The agent responsible for the development of these subcapsular hyaline necroses is evidently powerful, for these lesions are merely an exaggeration of the degenerative changes constantly present in this situation.

Pigmented Lesions.—The pathogenesis of the small nodular collections of pigment lying within and against the portal radicles and areas of organized hyaline necrosis in the late stages of stasis is conjecturable. They may possibly be due to interference with the delivery of bilirubin from the lobule by the interposition of a barrier of fibrous tissue which interrupts the continuity of the canaliculi with the bile ducts and that of the perivascular tissue spaces with the lymphatic vessels.

Infarction.—The occurrence in two cats, of multiple areas of infarction associated with thrombosis of branches of the portal vein and hepatic artery and with enormous distention of the bile ducts within the area of involvement serves to emphasize the importance of biliary stasis as a contributory factor in the development of this lesion. Cameron and Oakley expressed the belief that infection plays an important rôle in the production of infarcts following ligation of the common bile duct in rats. In the absence of bacteriologic data, we are unable to corroborate or deny this hypothesis, although no unusual inflammatory changes were present in the livers and biliary passages of the animals in which these lesions were observed.

Bile Duct and Connective Tissue Changes.—In the early stages of stasis there is an intimate relationship between the proliferation of bile ducts and connective tissue, although the latter is relatively much less marked in the first fifteen days. In the later stages, with the subsequent organization of the hyaline necroses about the portal radicles, this relationship is reversed; the increase of avascular connective tissue becomes enormous, pinching off and obliterating many of the vascular channels and the majority of the bile ducts which had proliferated previously.

SUMMARY

Comparison of Biliary Stasis in Man and in the Cat.—Although the hepatic changes associated with biliary stasis are specific for each species, there is a remarkable parallelism between the lesions in man and in the cat. In both, there is a tremendous volumetric increase in the biliary conducting system, with stretching and thinning of the walls; the process extends into the smaller branches, although the extrahepatic ducts show the greatest degree of involvement. The biliary conducting system, with the gallbladder in situ, contains dark, thick, ropy bile which later tends to become paler. The canaliculi are distended with biliary thrombi, many of which are extruded into the perivascular tissue spaces and sinusoids to be phagocytosed by Kupffer cells and macrophages. Bile

pigment, in the form of granules and droplets and in colloidal suspension, is present in varying amounts, chiefly in the cells of the central portion of the lobule. In the cat, however, white bile may form relatively rapidly and intrahepatic pigmentation in any form occurs less constantly and is less well defined than in man. In both, nonpigmented focal midzonal areas of necrosis occur and regressive changes involve the hepatic cells diffusely, although they are most marked immediately about the central vein. The hyaline type of necrosis is not observed in man; on the other hand, the only lesions in cats comparable to the biliary necroses seen in man are the collections of pigment within and around the thickened portal radicles in the late stages of stasis. The newly formed connective tissue, which proliferates independently in the later stages in both species, may have a periportal, interlobular, intralobular or, in relatively long-standing cases, even a perilobular distribution. Regeneration of hepatic cells is consistently a marked feature in the cat until the very late stages but apparently does not occur in complete and permanent stasis in man.

FOCAL CALCIFICATION OF THE BRAIN AND DURA OF A HYDROCEPHALIC IDIOT CHILD

GEORGE RUKSTINAT, M.D.
CHICAGO

Calcification of the brain substance with its accompanying vessels in localized regions is sufficiently infrequent to arouse comment especially when occurring in young children. The far-reaching effects of arteriosclerosis are well portrayed in Blackburn's [1] work on the brains of insane adults. In these both functional and anatomic changes were demonstrable. Detailed studies of calcification in cerebral vessels are recorded by Dürck [2] for epidemic encephalitis and by Weimann [3] for epilepsy. Bassoe and Hassin [4] encountered a localized indurated region in the brain of a young man who had been operated on repeatedly for a supposed tumor of the brain. The diseased region contained markedly calcified capillaries and was responsible for epileptic seizures. Weimann [3] believes that calcification of the brain without arteriosclerosis usually occurs from one of three causes: acute infections, as epidemic encephalitis or malaria; intoxications, as carbon monoxide poisoning, and various chronic diseases of the brain, as encephalitis interstitialis infantum (Virchow), epilepsy and cerebral atrophy. A casual search of the literature reveals an astounding amount of disagreement concerning such interpretations. What is regarded by some authors as a cause is listed by others as an effect. Thus, after studying the clinical and pathologic aspects of toxic encephalitis following acute infections of the respiratory tract, otitis media, acute mastoiditis, pneumonia, scarlet fever and septicemia in children, Grinker and Stone [5] found no evidence of microbic invasion of the brain. The stress of the toxic agent was apparently directed against the ganglion cells and vascular system; the changes resembled most those described by Hassin [6] for acute lead poisoning. Hassin concluded: "It seems permissible to claim that vascular infiltrative phenomena are indicative of an infectious type of encephalitis, while the proliferative

---

From the Norman Bridge Pathological Laboratory, Rush Medical College.

1. Blackburn, I. W.: Washington, D. C., Government Printing Office, 1908.
2. Dürck, H.: Ztschr. f. d. ges. Neurol. u. Psychiat. **72**:175, 1921; Verhandl. d. deutsch. path. Gesellsch. **18**:88, 1921.
3. Weimann, W.: Ztschr. f. d. ges. Neurol. u. Psychiat. **76**:533, 1922.
4. Bassoe, P., and Hassin, G.: Arch. Neurol. & Psychiat. **6**:359, 1921.
5. Grinker, R. R., and Stone, T. T.: Arch. Neurol. & Psychiat. **20**:244, 1928.
6. Hassin, G.: Arch. Neurol. & Psychiat. **6**:268, 1921.

phenomena denote a toxic condition." Grinker suggested that "severe neurologic sequelae may develop from acute toxic encephalitis associated with acute infections if recovery occurs, and that more attention should be directed to encephalitis as an etiologic factor in these sequelae. Rosenthal,[7] on the other hand, believed that he had evidence that old calcified vascular and parenchymal changes, ostensibly the result of trauma sustained at birth, supply the necessary susceptibility for acute encephalitis. He concluded:

> It is suggested that many of the idiopathic and atypical forms of encephalitis in children are due to the superimposition of a mild infection or intoxication on foci of lowered resistance in the brain secondary to insults sustained at birth. Colloid deposits and deposits of calcium in the brains of children are explained as sequelae to injuries sustained at birth.

That injuries to the brain are common following birth is generally conceded and experimental, clinical and pathologic studies bear evidence of the interest they incite.[8] Especially remarkable in this respect are the studies of Schwartz [8b] and Capper.[8c] The latter reported bloody cerebrospinal fluid in a new-born infant delivered by cesarean section. Such hemorrhage must be interpreted as indicating vascular damage in some part of the nervous system, but it is difficult to predict the extent of the injury. The probability of calcific depositions occurring at the sites of injury is still more difficult to predict. The recent interesting report of Courville and Kimball bears out this statement. These investigators [9] studied the histologic changes in a twenty-two year old gunshot wound of the brain and came to some interesting conclusions. They found that:

> Morphologically, crippled nerve cells may persist in the margins of wounds of the brain for many years. Nerve cells may, and usually do, persist in areas of cortex isolated by laceration or hemorrhage.
>
> Even after a prolonged interval the larger nerve fibers continue to undergo regressive change at the margin of the wounds of the brain. Both the myelin sheaths and the axis cylinders are involved in this process of disintegration. . . .
>
> Evidences of a persistent degenerative change were further indicated by the occurrence of free fat, yellow pigment and argentophilic material in wandering macrophages and the walls of the blood vessels, and by characteristic morphologic changes in the microglia in the adjacent cortex.
>
> A solid cicatrix was not observed.

There was no calcification anywhere along the extensive path of the bullet.

---

7. Rosenthal, S. R.: Arch. Path. **16**:33, 1933.

8. (*a*) Wohlwill, F.: Ztschr. f. d. ges. Neurol. u. Psychiat. **68**:384, 1921. (*b*) Schwartz, P.: Ibid. **90**:263, 1924. (*c*) Capper, A.: Am. J. Obst. & Gynec. **18**:106, 1929. (*d*) Roberts, M. H.: Am. J. Dis. Child. **38**:1196, 1929. (*e*) Tyson, R. M., and Crawford, W. H.: Am. J. Obst. & Gynec. **21**:694, 1931. (*f*) Ceelen, W.: Virchows Arch. f. path. Anat. **227**:152, 1920.

9. Courville, C. B., and Kimball, T. S.: Arch. Path. **17**:10, 1934.

In the third group of illnesses listed by Weimann [3] as preceding calcification of the brain is the much debated encephalitis congenita (Virchow). The local fatty changes in the brain originally thought degenerative by Virchow [10] in 1867 were regarded by Jastrowitz [11] as normal fat depots necessary to myelination. Partial accord was reached by these authors in 1883 when Virchow [12] withdrew his original statement concerning the inflammatory nature of these fat nodules but still maintained that the process was progressive. The renewal of interest in this subject some forty years later has been commented on by various authors [13] and a lack of complete accord is still apparent.

The difficulty of ascribing to some specific injury the calcification which eventually occurs is not mitigated by present concepts of dystrophic calcification. Such is the impression gained from Barr's [14] excellent review of the subject and from Wells' [15] interpretation of the events leading to calcification in arteries. Barr said:

> The tissues involved are of widely different origin and chemical character and seem to have in common only the quality of homogeneity and a physical appearance frequently resembling the hyaline cartilage in which new bones are formed. While fibrous and elastic tissues are most often involved, dystrophic calcification may also occur in degenerated ganglion cells of the brain. . . . Evidence indicates that dystrophic calcification is dependent on local conditions and that it is not initiated although it may be modified by general changes in calcium metabolism or in the state or amount of calcium in the circulating blood.

He reviewed the theories of calcium deposition in detail but necessarily left an impression of uncertainty about the process.

The homogeneous material in which calcium is eventually deposited seems to have a counterpart in the so-called pseudocalcium deposits frequently noted in the brains of normal persons and in those dying of a vast variety of diseases. These deposits are most plentiful in the putamen and globus pallidus and were originally believed to be the cause of chorea, paralysis agitans, tetany and many other conditions. Their association with certain endocrine disturbances, notably thyroprival tetany and Addison's disease, has recently received comment from Ostertag.[16] In eight of fifty brains he found these concretions present abundantly in the cerebellum. Kodama [17] found fat in identical regions of the brain in which the pseudocalcium deposits occurred.

---

10. Virchow, R.: Virchows Arch. f. path. Anat. **38**:129, 1867.
11. Jastrowitz, M.: Arch. f. Psychiat. **3**:162, 1871.
12. Virchow, R.: Berl. klin. Wchnschr. **19**:706, 1883.
13. Rosenthal,[7] Wohlwill.[8a] Schwartz.[8b]
14. Barr, D. P.: Physiol. Rev. **12**:593, 1932.
15. Wells, H. G.: Arteriosclerosis: A Survey of the Problem, New York, The Macmillan Company, 1933, chap. 11.
16. Ostertag, B.: Virchows Arch. f. path. Anat. **275**:828, 1929.
17. Kodama, I.: Ztschr. f. d. ges. Neurol. u. Psychiat. **102**:236, 1926.

These deposits stain with sudan III, but are not doubly refractive or soluble in acetone, ether or chloroform or their mixtures. They have a marked affinity for hematoxylin stains but react negatively to von Kossa's and Röhl's tests for calcium. Wells,[15] in discussing the microchemistry of calcification, stressed the fact that:

> Decalcification of bone has very little influence on the staining with alum hematoxylin, which therefore cannot depend on the presence of calcium salts but seems to depend on the character of the ground substance in which the calcium is deposited and its mordanting with iron, chromium or aluminum. In other words, alum hematoxylin is not a stain for calcium salts. There is, however, no doubt that areas of pathological calcification often stain deeply with alum hematoxylin and there are reasons for thinking some of this may be due to the presence of free iron.

The rôle played by iron and other substances in the staining of calcium was stressed by Cameron [18] as follows:

> Some of the staining which is obtained in bone and pathological calcifications is due to iron and in some instances the whole of it is to be so explained. But iron is not always present and most of the hematoxylin reaction is due to the presence in bone, in cartilage and at the sites of pathological calcification of some special ground substance which stains directly with alum hematoxylin and with simple hematoxylin after mordanting with chromium or aluminum. This substance has presumably some intimate relationship with the deposition of calcium salts since it is connected with the necrosis which precedes pathological calcification as well as with the laying down of normal bone. And hematoxylin is a test for calcification only in so far as it demonstrates this accompanying substance.

Therefore, the vicissitudes besetting the path of one bent on demonstrating the presence of calcium in brain tissue are many. Estimates of the relative ages of various deposits are difficult except after intra vitam employment of alizarin, an obvious impossibility in most instances as it was in the following case.

REPORT OF A CASE

M. M., a girl, aged 3½ years, born after a moderately severe labor, developed poorly physically and was an idiot. A sister, three years older, had developed to about the same degree physically and mentally and then died. Neither child showed the stigmas usually associated with mongolian idiocy. The parents had submitted to exhaustive medical investigation and were found normal. No unusual foods or large doses of irradiated sterols had been employed in treating the children.

M. M. was markedly emaciated and died of bronchopneumonia. The necropsy, about eight hours after death, disclosed the following: The fontanels were closed, the cranial sutures distinct and the calvarium symmetrical. The top half of the dura was opaque and glistening and about twice normal thickness in its dorsal third. On the right side in the wall of the superior longitudinal sinus 3 cm. proximal to the torcular Herophili was a yellowish-gray firm mass, 15 mm. long and from 1 to 2 mm. thick. Its long axis was parallel to the sinus, and a small

18. Cameron, G. R.: J. Path. & Bact. **33**:929, 1930.

lateral projection lay in contact with one of the larger cerebral veins. Similar but smaller deposits occurred in the dorsal half of both cerebral hemispheres, especially at the junction of the cortex and medulla, and two such masses formed ridges 1 mm. wide and 12 mm. long beneath the lining of the lateral ventricles. The masses were especially abundant in the putamen and dentate nucleus and could

Low power photomicrograph illustrating the calcium deposits in the cerebellum and the absence of reaction in their vicinity.

be traced about the larger arteries in these structures. All the deposits were extremely firm and gritty with the exception of that in the dura, which was only moderately so. The lateral ventricles were enlarged from bilateral internal hydrocephalus so that their maximum cross-diameter was 7 cm. The larger arteries and veins of the brain, except those mentioned elsewhere, were unaltered. The leptomeninges of the entire top of the brain were edematous, the convolutions narrow and the sulci deep.

Microscopically the deposits noted as gritty contained calcium which occurred as globules in the brain tissue and as cords about the capillaries and in the adventitia and media of the arteries. The globules at times formed conglomerate mulberry-like masses and at others combined with short cords to form structures like a child's jack with bulbous ends on thinner crossing and interlacing bands. In some arteries a compressed lumen was lessened to the diameter of a red blood corpuscle. About the densest deposits there was a marked capillary proliferation but only a slight increase in glia. Most regions were remarkable for the absence of any inflammatory or other change in the adjacent tissues. The concretions designated as calcium stained deep blue-purple with hematoxylin and gave positive von Kossa and Röhl tests. Weak acid applied to unstained sections under a cover slip caused minute bubbles to appear. With sudan III staining, fat was disclosed in the peripheral parts of the mass in the dura only. The Turnbull blue reaction was negative for iron.

The other organs were altered only in ways compatible with death from bronchopneumonia. The thymus weighed only 8 gm.

COMMENT

The presence of the calcified material in the outer portions of vessels and in the perivascular spaces is evidence, in some measure, of the route taken by the substance predisposing to calcification. The nature of this substance was not apparent, but it seems possible to postulate some injury to the brain at birth or as a result of the hydrocephalus which supplied the necessary base material in which calcium later accumulated. The limitation of these deposits to the brain and dura suggests a local disturbance. The small stature of the child is compatible with some endocrine dysfunction no longer apparent. The occurrence of similar symptoms in sisters of about the same age is interesting from the standpoint of a possible birth trauma common to both.

The hydrocephalus associated with a markedly thickened dura, falx cerebri and tentorium cerebelli may represent a sequel of tentorial hemorrhage and tears so elaborately described by Beneke.[19] The increased thickness of these structures, especially near the junction of the falx cerebri and tentorium, may be of prime significance in damming back blood in the great vein of Galen. Dandy[20] was able to produce bilateral internal hydrocephalus experimentally by obstructing this vein near its origin.

SUMMARY

1. Focal calcification in the brain and thickened dura of an idiot child are described; injury of the head during birth and endocrine dysfunction are offered as possible explanations.

2. The suggestion is also made that internal hydrocephalus may have occurred as a result of venous stasis in the great vein of Galen causing superabundant secretion of cerebrospinal fluid.

19. Beneke, R.: Beitr. z. path. Anat. u. z. allg. Path. **84:** 551, 1930.
20. Dandy, W.: Ann. Surg. **70:**129, 1919.

EXPERIMENTAL SIDEROSIS: I

VALY MENKIN, M.D.
BOSTON

The view that the pigment hemosiderin is in all cases a derivative of hemoglobin is still widely accepted. This relationship exists, it is true, in a large number of instances. Furthermore, the prevalence of such a concept is not surprising when it is recalled that hemosiderin is an iron-containing substance and that more than two thirds of the iron of the human body is contained within red blood corpuscles. Nevertheless, the literature contains reports of observations to the effect that hemosiderin may exist without any evidence of degradation of hemoglobin. In hemochromatosis, a disease characterized by abundant deposition of hemosiderin, there is no evidence of red blood cell destruction, according to Sprunt.[1] This investigator maintained that the pigment may be formed independently of hemoglobin from the iron of cells during autolysis. Mallory,[2] on the other hand, views hemofuscin in hemochromatosis as the intermediary pigment in the formation of hemosiderin from hemoglobin. Whipple [3] suggested that hemosiderin may be the result of a change in the fundamental pigment metabolism of the organism rather than a product of the partial degradation of hemoglobin.

The chemical nature of hemosiderin is unknown. According to Wells,[4] its ready solubility in weak acids and its prompt reactivity with agents which stain inorganic iron are indications that it is not a very complex compound. Many years ago, Biondi [5] expressed the view that hemosiderin was likely either a hydrated oxide of iron or perhaps, under certain circumstances, an iron proteinate. Following repeated subcutaneous injections of a saccharated solution of ferric oxide, Strasser [6]

From the Department of Pathology, Harvard University Medical School, Boston.

This investigation was aided by several grants, as follows: William W. Wellington Memorial Research Fund, the DeLamar Mobile Research Fund and the National Research Council.

1. Sprunt, T. P.: Arch. Int. Med. **8**:75, 1911.

2. Mallory, F. B.: Arch. Int. Med. **37**:336, 1926.

3. Whipple, G. H.: Arch. Int. Med. **29**:711, 1922. Whipple, G. H., and Bradford, W. L.: Am. J. Dis. Child. **44**:336, 1932.

4. Wells, H. G.: Chemical Pathology, Philadelphia, W. B. Saunders Company, 1925.

5. Biondi, C.: Beitr. z. path. Anat. u. z. allg. Path. **18**:174, 1895.

6. Strasser, U.: Beitr. z. path. Anat. u. z. allg. Path. **70**:248, 1922.

found deposits of an iron-containing yellowish-brown pigment in the spleen of the guinea-pig. This observation led him to conclude that hemosiderin is probably a ferric oxide compound. In his extensive monograph on pigments, Hueck [7] advanced the view that hemosiderin is an inorganic iron compound loosely bound to fats and proteins.

Rous and Oliver [8] transfused rabbit blood into normal rabbits over a period of many months. As a result of the destructive effect on the injected foreign red cells, large quantities of hemoglobin were liberated into the circulation. A widespread siderosis, practically similar in distribution to that found in hemochromatosis, ensued. In these experiments the iron-containing pigment was obviously derived from the degradation of hemoglobin. I recently demonstrated [9] that in tuberculous and in normal rabbits repeated intravenous injections of dilute ferric chloride are followed by the deposition of a yellowish-brown pigment. This material, indistinguishable from hemosiderin by known tests, appeared chiefly within mononuclear phagocytes. The inference drawn in a preliminary report was that a state of hemosiderosis had been produced experimentally by the injections of an iron salt and that this condition was evidently not referable to the degradation of hemoglobin. As present evidences show that this pigment is not *necessarily* associated with hemoglobin degradation, it is proposed to name it "cytosiderin," a more accurate designation, under the circumstances, than the specific term "hemosiderin." This paper, as well as an accompanying one,[10] will present in detail experimental evidences substantiating such a concept.

PRODUCTION IN VIVO OF A HEMOSIDERIN-LIKE PIGMENT (CYTOSIDERIN)

The iron-containing pigment was observed during a study of tissues of tuberculous rabbits that had been given repeated intravenous injections of a solution containing 0.25 per cent of ferric chloride in distilled water.[11] The animals were given the injections for variable intervals of time ranging from less than two weeks to several months; individual dosages of the iron salt varied from 5 to 10 cc. Many of the rabbits studied had been previously inoculated with a virulent culture of bovine tubercle bacilli. A number of this group, including animals given injections of ferric chloride as well as nontreated ones, succumbed to the disease. To control the possible effect of the disease on pigment deposition, normal rabbits were repeatedly given injections of the ferric chloride solution. They were then killed, and their tissues were compared with those of normal, nontreated animals as to the presence of the pigment. The results in this series of experiments were

7. Hueck, W.: Beitr. z. path. Anat. u. z. allg. Path. **54**:68, 1912.

8. Rous, P., and Oliver, J.: J. Exper. Med. **28**:629, 1918.

9. Menkin, V.: Proc. Soc. Exper. Biol. & Med. **31**:755, 1934.

10. Menkin, V., and Talmadge, S. M.: Arch. Path., this issue, p. 61.

11. Menkin, V.: J. Exper. Med. **55**:101, 1932; **60**:463, 1934; Am. J. M. Sc. **185**:40, 1933.

identical with the findings in the tuberculous rabbits. The tissues fixed in a dilute solution of formaldehyde, U. S. P. (1:10) for microscopic section included in most cases: spleen, liver, lungs, bone marrow, kidneys and suprarenal glands. Samples of these organs were also tested in the gross for the presence of iron by dipping them in acidified potassium ferrocyanide (prussian blue reaction). Microscopic sections were stained for iron by the same method; in addition, the ordinary hematoxylin and eosin preparations were studied.

The tissues of rabbits that had received no iron gave a completely negative prussian blue reaction, except in the case of the spleen, which frequently yielded a positive reaction. On the other hand, freshly removed organs of iron-treated animals, including the spleen, liver, bone marrow and kidneys, almost always revealed pronounced deposition of the metal when placed in acidified potassium ferrocyanide. The lungs when tested similarly in the gross failed to give the reaction for iron. These findings are in accord with the observations of previous investigators.[12]

The microscopic studies were of particular interest. The spleens of control animals which frequently yielded in the gross a definite reaction for iron in many instances showed no microscopic sign of iron either as pigmented or as nonpigmented material. The kidneys of experimental rabbits which almost invariably revealed in the gross large quantities of iron, particularly in the cortical region, seldom showed any sign of ferruginous deposition in microscopic preparations. The meager amount of iron-containing material seen in sections in the Kupffer cells of treated animals was in striking contrast to the extensive and diffuse prussian blue reaction throughout the parenchyma observed in the gross when the freshly removed organ was dipped in the testing reagent. There is some evidence that a large part of the iron is extracted during the technic of preparation for histologic study, presumably in the clearing process. These observations indicate that the injected iron evidently appears in two forms in tissues of animals: (1) as a nonpigmented substance, extracellularly situated in large part and apparently extracted to some extent during the clearing process and (2) as a pigmented material, located intracellularly and appearing as yellowish-brown, refractile granules, indistinguishable by known tests from hemosiderin. It is with the latter type of iron-containing substance that this study is primarily concerned.

The pigmented material was found in the spleen in practically all of the animals that had been treated repeatedly with ferric chloride, i. e., in twenty-eight of twenty-nine specimens studied (table). The material was, as a rule, located within the mononuclear phagocytes of the sinuses and reticular cords. The cells were often so loaded with

12. Boycott, A. E., and Price-Jones, C.: J. Path. & Bact. **17**:347, 1913. Muir, R., and Dunn, J. S.: J. Path. & Bact. **19**:417, 1914-1915.

this substance that the outlines of the cell wall could not at all times be discerned with any degree of certainty (fig. 1). Varying degrees of fibrosis, replacing in part the peripheral portions of the malpighian follicles, were frequently noted accompanying the deposition of the iron-containing pigment (fig. 2).

The results of all the experiments are summarized in the table. The number of plus signs serves as a rough indication of the amount of hemosiderin-like pigment found in the spleen, liver (Kupffer cells)

Distribution of Hemosiderin After Administration of Ferric Chloride

Control Animals				Experimental Animals				
	Amount of Hemosiderin Present				Amount of Ferric Chloride Injected, Cc.	Amount of Hemosiderin Present		
Rabbit Number	Spleen	Liver	Bone Marrow	Rabbit Number		Spleen	Liver	Bone Marrow
2-02	0	0		2-07	142	+	Trace	+
81	Trace	0		2-05	225	+		
2-06	0	0		2-85	226	+++	+	
2-04	++	0	0	2-84	233	+++	0	
2-14	++	0		2-13	238	+++	++	
3-00	0	0		3-25	280	++		
3-29	+	0		2-89	420	++	....	++
3-27	0	—		4-13	555	++	++	
3-35	—	—	0	3-11	130	0	0	
3-36	0	0		3-32	151	..	0	
3-10	0	0	0	3-33	180	+		
1-17	0	0	0	3-30	240	+		
3-34	++	0		4-93	211	++	0	
3-14	0	0	0	4-64	183	+++	+	
4-06	0	0		4-18	191	+	0	
4-94	0	0		4-15	205	++	0	
4-16	—	0		4-61	128	++	Trace	
8-21	0	0	0	8-15	57	+	0	+
7-71	0	—		8-17	70	++	+	
7-31	0	0		7-60	201	++	+	
7-80	0	0		7-51	219	+	0	
7-81	Trace	—		7-59	214	+++	++	
5-97	+	—	0	7-30	218	+++	+	
6-26	0	0	—	7-79	202	+	0	Trace
6-02	Trace	—	—	6-07	232	++	0	
6-24	Trace	0	0	5-41	278	++	Trace	
6-28	0	0		6-16	278	++	+	++
4-19	0	0	—	5-96	278	++	0	+
9-09	0	0		8-66	78	+	..	+
9-06	0	0		74	60	+		

and bone marrow of control and experimental animals. The Kupffer cells showed the presence of iron-containing granular material in about half of the animals studied. In the liver cells themselves the pigmented granules were observed rather infrequently. In the present series the material was not detected in the Kupffer or the parenchymatous cells of control animals although Rous and others have observed normal rabbit livers containing red cells in various stages of degradation together with hemosiderin. In the bone marrow of experimental rabbits the yellow iron-containing granules were invariably present in the reticular cells (fig. 3). They were altogether absent in the bone marrow of the controls.

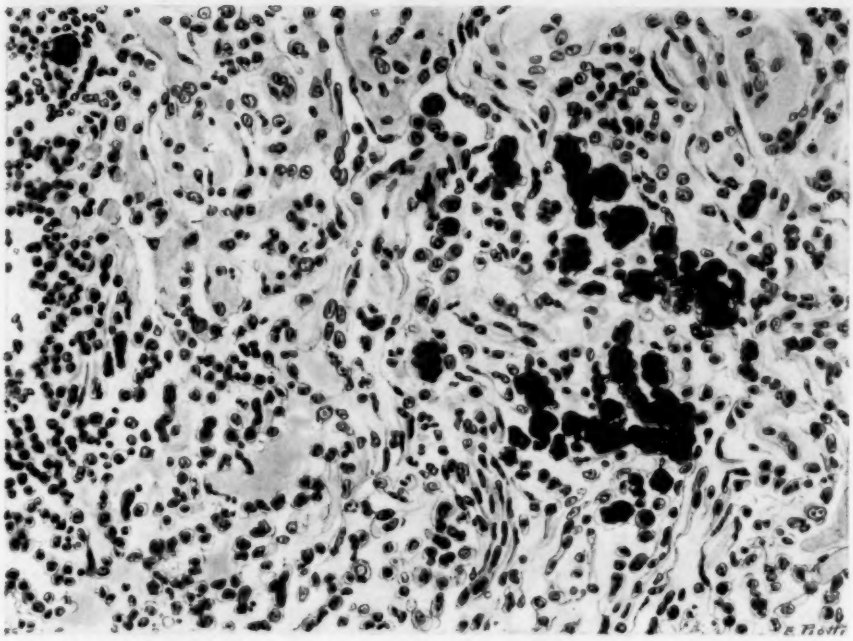


Fig. 1.—Drawing of a microscopic section through the spleen of a rabbit that had received a total of 218 cc. of ferric chloride intravenously. The large deposition of the iron-containing pigment cytosiderin is striking. (Reduced from a magnification of × 540.)

Fig. 2.—Photomicrograph of the spleen of a rabbit given repeated intravenous injections of dilute ferric chloride solution. In addition to the large collections (shown in black) of pigmented masses (cytosiderin), note the considerable amount of fibrosis replacing in part a malpighian follicle. (Reduced from a magnification of × 115.)

Brown [13] pointed out a number of years ago that hemosiderin-like products can be obtained experimentally by the injection of hematin derivatives. These substances he termed "hemosideroid," for he found that they were soluble in hydrogen dioxide or in potassium hydrate. True hemosiderin was insoluble in these reagents. When Brown's microchemical tests (using 3 and 30 per cent hydrogen dioxide or diluted potassium hydrate solution) were applied to the spleens of animals into which ferric chloride had been injected the pigment failed to dissolve, thus behaving like true hemosiderin.

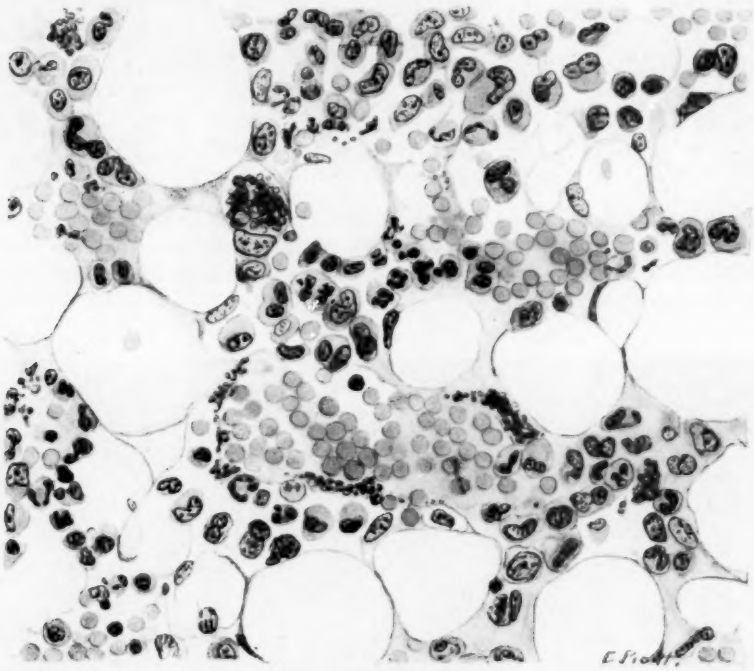


Fig. 3.—Drawing of a microscopic section through the bone marrow of a rabbit that had received a total of 420 cc. of ferric chloride intravenously. Note the abundance of granular pigment within the reticular cells. This material was found to contain iron (prussian blue reaction). (Magnification, × 760.)

The foregoing observations indicate that a state of hemosiderosis has been produced experimentally by the repeated intravenous injection of an inorganic iron salt. The blood picture findings, which are summarized in the accompanying paper, as well as the results of tissue culture studies to be described presently, indicate that the siderosis is not referable to the degradation of hemoglobin.

13. Brown, W. H.: J. Exper. Med. **14**:612, 1911.

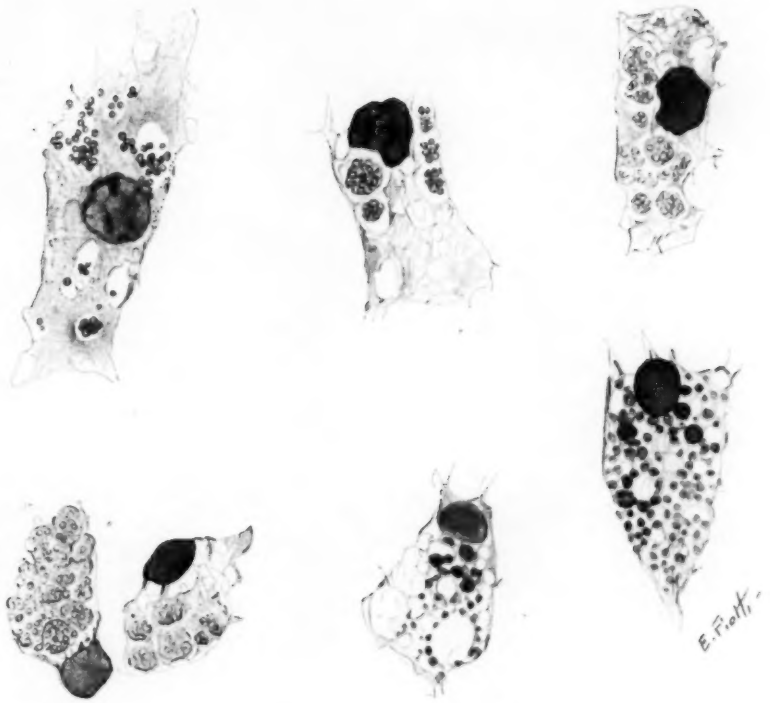


Fig. 4.—Drawing of mononuclear phagocytic cells taken from tissue cultures of lymph node explants (eight days old) in contact with ferric chloride. The intracellular deposits of the pigment cytosiderin are conspicuous. The iron-containing nature of this material is demonstrated in two of the cells (prussian blue reaction). (Magnification, × 1,300.)

PRODUCTION IN VITRO OF A HEMOSIDERIN-LIKE PIGMENT (CYTOSIDERIN)

Studies were undertaken to determine whether the iron-containing pigment obtained in the experiments just described following the administration of ferric chloride was the result of the direct action of cells on the iron salt rather than of breaking down of hemoglobin. The tissue culture technic offered a means of answering this question.

Under aseptic precautions mesenteric lymph nodes were removed from fairly young guinea-pigs. This tissue was selected in order to avoid as far as possible the presence of red blood corpuscles. To each explant was added 1 or 2 drops of plasma containing 0.25 per cent ferric chloride. It has been pointed out previously [14] that the precipitate formed by the interaction of dilute ferric chloride with blood serum redissolves. The ferric chloride-plasma solution used was obtained by adding the ferric salt until the point of saturation was reached. In the control explants an additional 1 or 2 drops of Tyrode's solution was added to make the fluid content equal to that of the experimental preparations. Otherwise the technic followed was the usual one employing splenic extract and plasma. Transfers were made on either the fourth or the fifth day. From eight to ten days after the beginning of the experiment the explants were fixed in a dilute solution of formaldehyde, U. S. P. (1:10), and stained with hematoxylin and eosin. In addition, the microchemical test for iron (prussian blue reaction) was performed on duplicate sections of all the explants studied.

No difficulty was encountered in producing proliferation of cells in a medium containing ferric chloride. Extensive growth from the explant usually occurred; indeed this was at times even more prominent than in control tissues. Careful histologic examination of explants in contact with ferric chloride invariably showed a moderate number of large mononuclear phagocytes containing in their cytoplasm various amounts of yellowish-brown, refractile, granular material similar in every respect to hemosiderin. The pigment yielded a definite prussian blue reaction when tested for iron (fig. 4). The pigment was absent in the control explants. The iron reaction in the treated explants showed definitely more mononuclear phagocytes containing ferruginous granules than there were cells containing yellow pigment in the parallel hematoxylin-eosin preparations. Furthermore, in some mononuclears there was a coarsely granular, grayish-brown material not quite similar to the yellow hemosiderin-like granules found in a number of cells. These facts warrant the inference that there is doubtless a definite latent period between the ingestion of the iron within the mononuclear cell and the formation of typical hemosiderin-like material. This indicates that the pigment is a product of intracellular digestion. Since ferric chloride dissolved in plasma forms probably a ferric proteinate,[15] it is likely that the pigment is the result of intracellular activity on such a compound. Similar evidences that hemosiderin is doubtless the end-product of phagocytic

14. Menkin, V.: J. Exper. Med. **53**:171, 1931.

15. Smythe, C. V., and Schmidt, C. L. A.: J. Biol. Chem. **88**:241, 1930.

activity on a relatively simple iron compound was obtained by in vivo experimentation. An animal killed after only a few intravenous injections of ferric chloride showed considerable iron (prussian blue test) throughout the spleen, whereas the amount of intracellular pigmented material was meager. When a long time elapsed between the administration of the iron salt and the death of the animal the converse tended to be true. Furthermore, in order to ascertain whether the pigmented material obtained in vitro reacts as true hemosiderin, several explants were treated with 30 per cent hydrogen dioxide for about thirty minutes. The pigmented material was evidently not destroyed by this treatment, for a number of cells were found that contained the brownish pigment, which yielded the typical reaction for iron.

COMMENT AND CONCLUSIONS

The observations reported indicate that an iron-containing pigment indistinguishable by known criteria from hemosiderin has been produced experimentally by the repeated intravenous administration of ferric chloride. The pigment shows fairly wide distribution but is found in greatest abundance within the mononuclear phagocytes of the spleen and bone marrow. It appears fairly often in the Kupffer cells of the liver but is rarely present in mononuclear cells of other tissues examined, such as the lungs or the suprarenal medulla. The iron-containing pigment reacts as true hemosiderin in that it is not dissolved by hydrogen dioxide or potassium hydrate. Spectroscopic analysis of these pigments will doubtless show whether there are subtle chemical differences between them which are indiscernible by the usual histopathologic procedures. The importance and possible applications of these studies, which are now in progress, are fairly obvious without further comment.

The iron-containing pigment can be produced in vitro by culturing lymph nodes in contact with the ferric salt. These observations, in addition to evidences presented in the accompanying paper, show that the production of iron-containing pigment is doubtless not referable solely to hemoglobin degradation but may also be the direct result of intracellular digestion of phagocytosed iron material present in the medium surrounding mononuclear phagocytes. In this connection it may be recalled that iron-containing pigment has been described in other cells, e. g., fibroblasts, liver cells and kidney tubules.[16]

"Cytosiderin" is proposed as a more inclusive and far more accurate designation for iron-containing pigments than "hemosiderin."

16. Rous, P.: J. Exper. Med. **28**:645, 1918.

EXPERIMENTAL SIDEROSIS

II. IRON-CONTAINING PIGMENT IN ABSENCE OF BREAKDOWN OF HEMOGLOBIN

VALY MENKIN, M.D.
AND
S. M. TALMADGE, B.S.
BOSTON

The production of an iron-containing pigment following repeated intravenous injections of ferric chloride was described in preceding communications.[1] Though the pigment was indistinguishable from hemosiderin, the name "cytosiderin" was proposed for it. The reasons for considering this a more inclusive and accurate designation for iron-containing pigments were mentioned. The view was advanced that such pigmented material is most likely the end-product of intracellular digestion of relatively simple iron compounds. Strong support for this concept was obtained in the formation of the pigment within mononuclear phagocytes cultivated in vitro in contact with plasma containing ferric chloride.

The object of this brief report is to present additional evidence showing that whatever influence ferric chloride may have on the breakdown of hemoglobin, this doubtless plays an insignificant rôle in the formation of cytosiderin.

EXPERIMENTAL WORK

Effect of Ferric Chloride on the Blood Picture.—Several rabbits received repeated intravenous injections of a 0.25 per cent solution of ferric chloride in distilled water. The course of injections was maintained for several months. Prior to the onset of the administration of ferric chloride and at various intervals throughout the course of the intravenous injections, red blood cell counts, hemoglobin determinations (Hellige method) and blood smears, stained by Wright's method, were obtained. Two rabbits not receiving injections served as controls.

The data are summarized in table 1. It is clear that injections of ferric chloride produced no appreciable effects on the number of red blood cells or the percentage of hemoglobin. There was no evidence of anemia, nor did the blood smears show abnormalities. Changes in the reticulocyte counts were not followed up, but the counts in eight animals that had

---

From the Department of Pathology, Harvard University Medical School.

This investigation was aided by grants from the National Research Council and from the Committee on Therapeutic Research, American Medical Association.

1. Menkin, V.: Arch. Path., this issue, p. 53; Proc. Soc. Exper. Biol. & Med. **31**:755, 1934.

received repeated injections of the ferric salt showed an average of 1.2 per cent reticulocytes, the individual counts ranging from 0.3 to 1.9 per cent. This is well within the normal limits. Furthermore, nine nontreated rabbits gave reticulocyte counts ranging from 0 to 2.3 per cent, with an average of 0.79 per cent. The difference between the average reticulocyte counts for the experimental and control animals is apparently negligible. The presence of a degree of anemia sufficient to account for the large deposits of iron-containing pigment in the tissue seems, therefore, out of the question. It is also to be noted that no microscopic evidence was obtained of a hyperplastic response on the part of the bone marrow as a result of the administration of the iron salt.

TABLE 1.—*Effect of Injections of Ferric Chloride ($FeCl_3$) on the Number of Red Blood Corpuscles and on the Percentage of Hemoglobin*

	Before Administration of $FeCl_3$		After Intravenous Administration of Ferric Chloride for Given Period											
			12 Days			26 Days			87 Days			132 Days		
Rabbit	Red Blood Cells, Millions per C.Mm.	Hemoglobin, per Cent	Red Blood Cells, Millions per C.Mm.	Hemoglobin, per Cent	Total Amount $FeCl_3$ Injected, Cc.	Red Blood Cells, Millions per C.Mm.	Hemoglobin, per Cent	Total Amount $FeCl_3$ Injected, Cc.	Red Blood Cells, Millions per C.Mm.	Hemoglobin, per Cent	Total Amount $FeCl_3$ Injected, Cc.	Red Blood Cells, Millions per C.Mm.	Hemoglobin, per Cent	Total Amount $FeCl_3$ Injected, Cc.
7-14 Exp.	6.43	102	6.94	97	16	6.28	105	41	6.30	102	146	6.1	105	206
7-36 Exp.	6.72	102	6.42	100	16	6.44	98	41	6.65	100	146	6.45	104	206
7-74 Exp.	6.87	98	7.09	102	16	6.08	91	41	6.12	105	146	7.1	93	213
7-90 Con.	6.51	90	6.45	104	..	7.30	98	..	6.24	100				
7-63*Con.	6.38	94	6.98	88	..	5.36	74							

* This animal succumbed seventy-eight days after the onset of the experiment.

Hemolytic Effect of a 0.25 Per Cent Solution of Ferric Chloride.—In order to ascertain whether the intravenous injection of a 0.25 per cent solution of ferric chloride in distilled water (isotonic with a 0.17 per cent solution of sodium chloride) produces a hemolytic effect that may ultimately account for the formation of pigment, the following experiments were performed.

Blood from the marginal ear vein of the rabbit was obtained in a test tube containing a saline solution of heparin. Immediately following this, from 5 to 10 cc. of a 0.25 per cent solution of ferric chloride was introduced into the vein of the opposite ear. At an interval of from several minutes to one-half hour later, a sample of blood was taken from the ear that had been bled first. Both samples of blood were centrifugated for about ten minutes and the supernatant plasma examined for hemolysis.

The results of these experiments appear in table 2. It is seen that the plasma of the blood removed after the injection of ferric chloride

invariably revealed considerable amounts of hemolysis. It was usually tinged a cherry-wine color, in marked contrast to the straw-colored or faintly tinged samples obtained from controls. This hemolytic effect was doubtless due to the introduction into the ear vein of a relatively large volume of a hypotonic solution of ferric chloride. Several hours subsequent to intravascular injection of the iron salt, however, there was no longer any evidence of hemolysis in the plasma. The immediate effect of the initial laking on the number of red blood corpuscles was not detectable by the usual counting chamber technic. Samples of blood were examined at frequent intervals for a period of several hours following the injection of the ferric chloride, but not even a transitory reduction in the number of red cells was discerned. It is conceivable, however, that hemolysis of a relatively small number of corpuscles

TABLE 2.—*Hemolysis of Red Cells Induced by the Intravenous Injection of a 0.25 Per Cent Solution of Ferric Chloride ($FeCl_3$) in Distilled Water*

Rabbit	Presence of Laked Red Blood Corpuscles in Plasma	
	Before Intravenous Injection of 0.25 per Cent $FeCl_3$ in Distilled H_2O	After Intravenous Injection of 0.25 per Cent $FeCl_3$ in Distilled H_2O
7-74	0	+++
8-52	Trace	+ to ++
9-15	Trace	+
9-16	Faint trace	++
8-47	Trace	++

might gradually, on repeated injections of the salt, cause a fairly conspicuous siderosis. For this reason the following experiments were undertaken.

Effect of an Isotonic 0.25 Per Cent Solution of Ferric Chloride on Red Blood Cells and Formation of Pigment.—A 0.25 per cent solution of ferric chloride, isotonic with a physiologic saline solution, was prepared by adding 250 mg. of the iron salt and 680 mg. of sodium chloride to 100 cc. of distilled water. When this solution was injected into the circulation of a rabbit, there was no evidence of hemolysis, even though samples of plasma were examined almost immediately after injection of the isotonic ferric solution. Likewise, the addition of the solution to blood in vitro failed to produce any hemolytic change.

The results of several experiments of this type appear in table 3. The question arises as to whether repeated injections of this nonhemolyzing solution of ferric chloride result in the deposition of cytosiderin. An experiment of this kind would serve to determine the rôle of the slight degree of hemolysis as a factor in the formation of the iron-containing pigment. Consequently, two rabbits received daily intravenous injections of from 5 to 10 cc. of the isotonic solution of ferric chloride. One animal was killed after a total of 65 cc. of the solution of the iron salt had been given over a period of thirteen days; the other continued

to receive injections until a total of 125 cc. had been administered. In both these rabbits a very extensive siderosis was observed within the mononuclear phagocytes of the spleen and bone marrow. The iron-containing pigment was also found to some extent in the Kupffer cells of the liver. Furthermore, it is interesting to note that when a 0.17 per cent solution of sodium chloride was injected intravenously a slight degree of hemolysis occurred in the plasma. This physiologically hypotonic solution is isotonic with a 0.25 per cent solution of ferric chloride in distilled water. Yet when a rabbit received repeated intravenous injections of from 5 to 10 cc. of this hypotonic saline solution totaling 126 cc., a study of the tissues failed to reveal any trace of cytosiderin. These experiments evidently demonstrated that the formation of iron-containing pigment was referable to the introduction of the ferric salt per se, rather than to the degradation of hemoglobin released as a result of hemolysis.

TABLE 3.—*Effect on the Red Cells of Intravenous Injection of a 0.25 Per Cent Solution of Ferric Chloride ($FeCl_3$) Rendered Isotonic With a 0.68 Per Cent Solution of Sodium Chloride*

	Presence of Laked Red Blood Corpuscles in Plasma	
Rabbit	Before Intravenous Injection of Isotonic $FeCl_3$	After Intravenous Injection of Isotonic $FeCl_3$
9-95	0	0
9-98	0	0
9-98	0	0
9-17	0	0

When several cubic centimeters of a 0.25 per cent solution of ferric chloride in distilled water was added to an approximately equal, or even a slightly greater, volume of whole blood, immediate hemolysis took place and the hemoglobin was changed into methemoglobin. Experiments in vitro with ferric chloride have shown that hemolysis always precedes the formation of methemoglobin. When an isotonic solution of ferric chloride, for example, is added to red blood corpuscles, neither hemolysis nor formation of methemoglobin occurs; but if to such a suspension a sufficient quantity of distilled water is added, laking of corpuscles follows and methemoglobin is produced. This conversion of hemoglobin into methemoglobin by ferric chloride may have points of theoretical interest. Several years ago, Conant [2] pointed out that perhaps hemoglobin should be regarded as a complex ferrous salt, somewhat in the nature of potassium ferrocyanide. This concept of hemoglobin is now generally accepted. Küster [3] expressed the opinion that whereas in the hemoglobin molecule the iron is in the ferrous state, in methemo-

2. Conant, J. B.: J. Biol. Chem. **57**:401, 1923.
3. Küster, W.: Ztschr. f. physiol. Chem. **66**:244, 1910.

globin it exists in the ferric form. The oxidation of hemoglobin to methemoglobin by the addition of ferric chloride and the consequent reduction of the latter, therefore, renders it possible that the iron salt may be carried in the circulation, at least in part, as a reduced ferrous salt. Whether the effectiveness of injections of ferric chloride in experimental tuberculosis, demonstrated in earlier studies,[4] bears any relation to the change of the iron from the ferric to the ferrous state remains to be seen. Conant and his pupils [5] have shown that the hemoglobin-methemoglobin system is really a reversible oxidation-reduction reaction. This investigator pointed out that the body has the capacity of reducing methemoglobin back to hemoglobin. The fact that samples of blood taken from animals receiving for many weeks injections of a hypotonic solution of ferric chloride fail to show any gross sign at least of the formation of methemoglobin seems to be in support of this view. Production of methemoglobin is hardly a factor in the deposition of cytosiderin. This is quite evident for when the injected solution of the iron salt is isotonic and therefore nonhemolytic, the formation of methemoglobin, under these circumstances, is precluded, as previously pointed out, and yet the pigment is deposited in abundance.

CONCLUSIONS

Whereas repeated intravenous injections of a 0.25 per cent solution of ferric chloride in distilled water are followed by extensive siderosis, there is no appreciable change in the number of red blood cells, percentage of hemoglobin or reticulocyte count.

Since this solution of ferric chloride is hypotonic, its injection into the circulation induces a certain amount of hemolysis. However, the laking action evidently plays an insignificant rôle in the formation of iron-containing pigment, for when a nonhemolytic, isotonic solution of ferric chloride is substituted for the hypotonic one, the same type of deposition of cytosiderin occurs. Furthermore, when a hypotonic saline solution is repeatedly injected intravenously, iron-containing pigment fails to be deposited.

These experiments furnish additional evidence that iron-containing pigment (indistinguishable from hemosiderin) need not be derived *solely* through the action of phagocytes on the products of the degradation of hemoglobin, but may arise also as a result of the intracellular digestion of available iron-containing material, exogenous as well as endogenous.

4. Menkin, V.: Am. J. M. Sc. **185**:40, 1933.

5. Conant, J. B.: The Harvey Lectures, 1932-1933, Baltimore, Williams & Wilkins Company, 1934, p. 159.

EFFECTS OF ARTIFICIALLY INDUCED LYMPHOPENIA IN CANCER-RESISTANT AND CANCER-SUSCEPTIBLE RATS

M. F. GUYER, PH.D., LL.D.
F. E. MOHS, M.D.
AND
E. M. SHEBESTA, M.D.
MADISON, WIS.

A rôle in resistance against cancer has been ascribed to the lymphocyte by a number of workers, but this theory has been denied by many others. In order to provide more evidence regarding the part played by lymphocytes in resistance to cancer and particularly to determine whether or not they are responsible for the resistance shown by our own cancer-resistant strains of rats, the present series of experiments were carried out.

By means of selective breeding of cancer-resistant rats we have been able to isolate from our ordinary laboratory stock of rats, which is about 85 per cent susceptible to transplanted Flexner-Jobling rat carcinoma, a strain which is highly resistant. For example, in the seventh selected generation only fifteen of one hundred and twenty-eight rats (11.7 per cent) showed a perceptible growth of the tumor at the end of three weeks—the time set arbitrarily for designating the animals as susceptible or resistant to the implanted tumor—while in the eighth generation only six of ninety-three rats (6.4 per cent) showed a growth. However, an even greater resistance than is indicated by these figures exists because in the total two hundred and twenty-one rats of the seventh and eighth generations the tumors grew progressively in only two (less than 1 per cent); in the others they ultimately underwent complete regression. Having thus isolated a strain of rats which were otherwise presumably the same as the original 85 per cent susceptible stock, we thought it desirable to determine, if possible, the nature of this inherited resistance.

Among proponents of the theory that the lymphocyte is a factor in resistance to cancer Da Fano [1] was the first to propose that the lymphocytes seen around degenerating tumors might be the agent which

This investigation was supported by a research grant from Mary C. Brittingham.

From the Department of Zoology and the Department of Radiology and Physical Therapy, University of Wisconsin.

1. Da Fano, C.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **5**:1, 1910.

distributes immunity throughout the body. Burgess [2] ascribed the regression of implanted tumors to impairment of their nutrition by the surrounding cellular exudate. Tyzzer,[3] Woglum [4] and others have also described the lymphocytic infiltration seen around regressing tumors. Baeslack [5] demonstrated that in mice with rapidly growing tumors the lymphocytes in the blood decreased and the polymorphonuclears increased, while in mice with regressing tumors the lymphocytes increased and the polymorphonuclears decreased. This inverse ratio has also been found to obtain in thirty-five of fifty (65 per cent) rats in which Flexner-Jobling carcinoma was implanted in our own laboratory (unpublished data). Murphy and Morton [6] showed that in mice with natural or induced immunity a marked lymphocytosis occurred on cancer implantation and that when this lymphocytosis was prevented by large doses of roentgen rays the immunity was destroyed. They [7] further showed that stimulation of the lymphoid organs by small doses of roentgen rays increased the resistance of mice to cancer.

Evidence against the lymphocyte as a factor in resistance to cancer has been presented by Sittenfield,[8] who found that lymphocytosis did not inhibit Flexner-Jobling carcinoma and that reduction of the lymphocytes by roentgen rays did not increase the susceptibility of cancer-resistant rats. Later he [9] was unable to increase the resistance to cancer by stimulating doses of roentgen rays in rats, but he had a measure of success in experimenting with Murphy's mice. Prime [10] was unable to increase the susceptibility of a strain of rats 90 per cent immune to Flexner-Jobling carcinoma by large doses of roentgen rays. His experiment was similar to the present series except for the fact that Prime's immune strain was apparently an entirely distinct stock whereas our immune animals have been secured by selection generation after generation from a highly susceptible (85 per cent) stock, and hence presumably are the same as the susceptible animals except for the immunity itself. The possibility of race differences which might mask the real defense mechanism are thus ruled out to a greater extent than in earlier experiments. Later Prime,[11] using his previous methods, was unable to increase the susceptibility of mice to transplanted spon-

2. Burgess, A. M.: J. M. Research **21**:575, 1909.
3. Tyzzer, E. E.: J. M. Research **32**:201, 1915.
4. Woglum, W. H.: J. Cancer Research **9**:171, 1925.
5. Baeslack, F. W.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **20**: 421, 1914.
6. Murphy, J. B., and Morton, J. J.: J. Exper. Med. **22**:204, 1915.
7. Murphy, J. B., and Morton, J. J.: J. Exper. Med. **22**:800, 1915.
8. Sittenfield, M. J.: J. Cancer Research **2**:151, 1917.
9. Sittenfield, M. J.: J. M. Research **38**:465, 1918.
10. Prime, F.: J. Cancer Research **5**:105, 1920.
11. Prime, F.: J. Cancer Research **6**:1, 1921.

taneous tumors. His results, however, were in contrast to those obtained by Chambers, Scott and Russ[12] in a similar experiment. Kellert[13] found that the high content of lymphocytes in the peritoneal fluid caused no inhibition of sarcoma 180 planted intraperitoneally.

EXPERIMENTAL PROCEDURE

The technical factors used in irradiation were: Westinghouse air-cooled 200 kilovolt deep therapy tube, full wave rectification, 160 kilovolts, 3 milliamperes, 2 mm. aluminum filter, 50 cm., focal skin distance, tube output 18 roentgens (r) per minute measured in air, effective wavelength 0.31 angstrom and exposure to the entire dorsum of the body.

EXERIMENT 1.—This was a preliminary experiment to determine how large a dose the rats could tolerate. Four immune rats were used. They were given 100, 200, 300 and 400 r, respectively, and then each was given 400 r on the third

TABLE 1.—*Experiment 2: Effect of Irradiation on Immune Rats*

Rat	Cell Count*	Initial Count	Count on 6th Day	Fate of Cancer
1	A	9,850	2,600	Regressed in 3 wks.
	B	6,895	1,976	
	C	70%	76%	
2	A	18,000	3,350	Regressed in 3 wks.
	B	14,760	2,546	
	C	82%	76%	
3	A	12,800	4,450	Regressed in 3 wks.
	B	8,832	3,649	
	C	69%	82%	
4	A	15,650	3,150	Regressed in 3 wks.
	B	12,368	2,425	
	C	79%	77%	

* A shows the total leukocyte count; B, the number of lymphocytes; C, the percentage of lymphocytes.

and sixth days of the experiment. Differential counts only were made in this experiment; they were done initially and on the fifth and eighth days. No significant change occurred in the differential count although the leukopenia was evident. The rats failed rapidly after the first week and were all dead by three weeks. This clearly was too great a dosage.

EXPERIMENT 2.—Four immune rats were used. Total and differential counts were made initially and on the sixth day. The rats were given 200 r on the first and third days. Cancers were implanted on the eighth day. The results are given in table 1.

It is apparent that the leukocytes were markedly depressed by the total of 400 r, but the percentages of lymphocytes remained practically the same, indicating that no selective destruction of lymphocytes occurred. The outstanding fact is that the rats retained their immunity notwithstanding the great decrease in the lymphocytes.

12. Chambers, H.; Scott, G., and Russ, S.: J. Path. & Bact. **23**:384, 1920.
13. Kellert, E.: J. Cancer Research **6**:41, 1921.

EXPERIMENT 3.—In order to ascertain whether smaller doses administered over a longer period resulted in a more selective destruction of the lymphocytes four immune rats were given four 100 r doses at forty-eight hour intervals. Complete leukocyte counts were made initially and on the tenth day. Cancers were implanted on the tenth day. The results are given in table 2.

TABLE 2.—*Experiment 3: Effect of Irradiation on Immune Rats*

Rat	Cell Count*	Initial Count	Count on 10th Day	Fate of Cancer
1	A	14,610	12,400	Regressed in 4 wks.
	B	11,395	11,284	
	C	78%	91%	
2	A	19,750	6,300	Regressed in 3 wks.
	B	16,587	5,670	
	C	83%	90%	
3	A	15,600	10,550	Regressed in 3 wks.
	B	12,012	8,123	
	C	77%	77%	
4	A	16,800	10,700	Regressed in 3 wks.
	B	10,752	9,630	
	C	64%	90%	

* A shows the total leukocyte count; B, the number of lymphocytes; C, the percentage of lymphocytes.

TABLE 3.—*Experiment 4: Effect of Irradiation on Immune and on Susceptible Rats*

Immune Rats	Cell Count*	Initial Count	Count on 11th Day	Count on 21st Day†	Count on 31st Day	Count on 50th Day	Fate of Cancer
Rat 1	A	17,400	13,100	7,700	11,450	17,450	Regressed in 3 wks.
	B	14,790	7,336	4,466	7,542	13,611	
	C	85%	56%	58%	65%	78%	
Rat 2	A	14,050	8,300	4,700	10,650	13,850	Regressed in 3 wks.
	B	10,678	4,897	2,491	6,603	8,856	
	C	76%	59%	53%	62%	64%	
Rat 3	A	10,350	6,500	12,650	30,100	27,050	Regressed in 4 wks.
	B	7,866	3,672	3,975	24,832	17,312	
	C	76%	56.5%	30%	82.5%	64%	
Rat 4	A	21,650	4,000	12,950	19,700	14,050	Regressed in 3 wks.
	B	16,887	3,080	9,583	7,387	9,413	
	C	78%	77%	74%	37.5%	67%	
Susceptible Rats							
Rat 5	A	12,700	4,650	3,475	.....	.....	Not planted
	B	11,938	3,580	2,519	.....	.....	
	C	94%	77%	72.5%	Dead	.....	
Rat 6	A	24,650	6,550	5,800	25,600	16,200	Grew progressively; 1 cm. at 3 wks.
	B	22,185	5,043	4,147	22,528	11,250	
	C	90%	77%	71.5%	88%	69.5%	
Rat 7	A	18,100	8,100	5,100	12,350	11,450	Regressed in 3 wks.
	B	13,575	4,779	2,703	9,015	8,244	
	C	75%	59%	53%	73%	72%	
Rat 8	A	15,900	7,000	4,700	.....	.....	Not planted
	B	13.833	3,640	2,444	.....	.....	
	C	87%	52%	52%	Dead	.....	

* A shows total leukocyte count; B, the number of lymphocytes; C, the percentage of lymphocytes.
† The last irradiation was on the twentieth day.

It is evident that the total count is depressed, but the relative percentage of lymphocytes is not decreased. Even the smaller doses of 100 r failed to cause selective destruction of the lymphocytes. Again, the rats remained immune despite the decrease in lymphocytes.

EXPERIMENT 4.—It was thought possible that still smaller doses might bring about the desired selective destruction of lymphocytes. Accordingly in this experiment 50 r was given every three days, and the blood counts were followed until the desired destruction of leukocytes was obtained. Six doses of 50 r or a total of 300 r was given, the last dose being administered on the twentieth day of the experiment. Cancer was implanted on the twenty-fifth day. Rats 1 to 4 were of the seventh generation of immune animals and rats 5 to 8 were from the susceptible stock. The results are given in tables 3 and 4.

These results clearly indicate that with the smaller doses spread over a longer period the lymphocytes were destroyed with some degree of selectivity since in the general leukopenia produced the percentage of lymphocytes in the total leukocyte count was noticeably less. The counts gradually rose again when irradiation was discontinued after the twentieth day, and by the fiftieth day of the experiment the counts were almost up to their initial level. Since all the cancers of the immune rats regressed it is evident that the reduction of the lymphocytes did not do away with the immunity of these animals.

TABLE 4.—*Experiment 4: Average Number of Lymphocytes*

Group	Average Initial Count	Count on 11th Day	Count on 21st Day	Count on 31st Day	Count on 50th Day
Immune rats	12,555	4,946	5,084	7,177*	12,208*
Susceptible rats	15,382	4,260	2,954	15,771	9,751
Immune and susceptible rats	13,868	4,504	4,019	12,984	11,448

* Rat 3 was omitted because of a leukocytosis resulting from a respiratory infection.

Judging from the much more rapid drop in their leukocyte counts the susceptible rats were more vulnerable than the immune to the destructive action of the roentgen rays on the leukogenic structures. Moreover, the general health and resistance of the susceptible rats to infection were more impaired than those of the immune animals. Susceptible rats 5 and 8 died of respiratory infections, not improbably as a result of the great reduction of the leukocytes. With one exception the immune rats apparently remained healthy.

EXPERIMENT 5.—The purpose of this experiment was to check the observations in experiment 4 with a larger number of rats. A total of twenty-four rats were used, twelve from the immune strain and twelve from the susceptible stock. After an initial cell count was made the rats were given doses of 50 r at intervals of three days, the cell counts being repeated every ten days throughout the experiment. After six doses the susceptible animals appeared rather weak and unhealthy; so the seventh dose was not given to that group. The animals recuperated several days later, and the final dose was given. Thus the susceptible rats received a total of 350 r and the immune, a total of 400 r. Cancers were implanted on the twenty-first day of the experiment. Nine susceptible control animals were also given implants.

In order to study the cellular reactions surrounding the cancers two tumors were implanted into each of the following: rats 1 and 2 of the immune group,

rats 1 and 2 of the susceptible group, two susceptible control and two immune control animals. Seven days after implantation one of the cancers from each of these rats was removed along with some overlying skin and surrounding tissue, and microscopic sections of the growths were made. The other cancers in these rats were left in place for further observation.

Tables 5 and 6 give the results of irradiation on the immune and susceptible groups, respectively.

TABLE 5.—*Experiment 5: Effect of Irradiation on Immune Rats*

Rat	Cell Count*	Initial Count	Count on 10th Day	Count on 17th Day	Count on 27th Day	Count on 37th Day	Fate of Cancer
1.....	A	19,300	9,850	7,150	14,000	10,150	Regressed in 3 wks. (4/14/34)
	B	15,150	7,141	4,754	10,780	9,236	
	C	78.5%	72.5%	66.5%	77%	91%	
2.....	A	14,250	6,400	6,450	9,900	10,150	Regressed in 3 wks.
	B	11,471	4,544	4,708	7,425	7,612	
	C	80.5%	71%	73%	75%	75%	
3.....	A	14,900	7,400	9,050	11,950	12,150	Regressed in 3 wks.
	B	10,950	5,439	7,873	10,396	10,084	
	C	73.5%	73.5%	87%	87%	83%	
4.....	A	14,950	9,200	9,250	18,300	11,450	Regressed in 3 wks.
	B	11,601	6,808	6,845	13,988	9,732	
	C	78%	74%	74%	76%	85%	
5.....	A	16,550	8,650	5,950	10,100	10,200	Regressed in 3 wks.
	B	14,867	6,314	4,284	6,918	8,058	
	C	89%	73%	72%	68.5%	79%	
6.....	A	11,900	10,050	9,600	11,450	11,900	Regressed in 3 wks.
	B	11,007	6,432	7,296	8,931	9,538	
	C	92.5%	64%	76%	78%	81.5%	
7.....	A	13,300	7,700	5,700	11,200	7,600	Regressed in 3 wks.
	B	11,637	5,890	4,350	8,176	6,160	
	C	87.5%	76.5%	76.5%	73%	80%	
8.....	A	15,000	9,400	5,600	11,650	12,200	Grew to 1.4 cm. in 3 wks. and then regressed slowly
	B	11,007	5,922	4,088	9,902	8,052	
	C	75%	63%	73%	85%	66%	
9.....	A	17,450	6,450	4,500	13,700	11,050	Grew to 1.4 cm. in 3 wks. and then regressed slowly
	B	15,007	4,934	3,240	11,371	6,961	
	C	86%	76.5%	72%	83%	63%	
10.....	A	10,850	8,650	7,650	15,200	8,500	Regressed in 3 wks.
	B	9,385	5,968	5,202	11,704	6,290	
	C	86.5%	69%	68%	77%	74%	
11.....	A	15,950	10,050	7,750	11,700	8,750	Regressed in 3 wks.
	B	12,281	6,231	6,510	9,360	6,125	
	C	77%	62%	84%	80%	70%	
12.....	A	14,500	10,450	7,400	13,000	13,050	Regressed in 3 wks.
	B	11,237	7,628	5,772	10,205	9,874	
	C	77.5%	73%	78%	78.5%	68%	
Average number of lymphocytes	B	12,133	6,104	5,410	9,935	8,143	All but 2 regressed in 3 wks.; the 2 exceptions regressed later
	C	81.5%	70.7%	75%	78.1%	76.3%	

* A shows the total leukocyte count; B, the number of lymphocytes; C, the percentage of lymphocytes.

It is evident that in all the cases there was a decided drop in the total leukocyte count, and also in most cases a disproportionately greater drop occurred in the percentage of lymphocytes. The counts gradually returned toward the initial level after irradiation was stopped, but that level had not been reached by the thirty-seventh day.

The fate of the cancers was not significantly altered in the strain of immune rats, there being two successful inoculations out of twelve

(16.6 per cent) at three weeks. The two implants though positive at three weeks subsequently regressed. In a series of one hundred and twenty-eight rats of the same (seventh) generation of immune animals not receiving irradiation there were sixteen positive implants (12.5 per cent) at three weeks.

TABLE 6.—*Experiment 5: Effect of Irradiation on Susceptible Rats*

Rat	Cell Count*	Initial Count	Count on 10th Day	Count on 17th Day	Count on 27th Day	Count on 37th Day	Fate of Cancer at Three Weeks
1	A	22,400	9,100	8,700	20,600	26,250	0.9 cm.
	B	19,488	7,007	5,655	17,716	20,343	
	C	87%	77%	65%	86%	77.5%	
2	A	31,400	9,800	6,200	11,700	22,350	1.7 cm.
	B	25,905	7,693	5,084	9,828	15,763	
	C	82.5%	78.5%	82%	84%	75.5%	
3	A	24,450	8,200	7,850	15,250	16,850	1.2 cm.
	B	20,294	6,724	6,790	14,030	12,637	
	C	83%	82%	86.5%	92%	75%	
4	A	12,750	8,550	6,100	14,100	13,200	Regressed
	B	7,905	5,643	4,392	10,293	11,088	
	C	62%	66%	72%	73%	84%	
5	A	15,150	8,100	11,700	18,750	17,600	1.0 cm.
	B	12,575	6,318	8,599	15,750	15,224	
	C	83%	78%	73.5%	84%	86.5%	
6	A	15,050	6,000	6,100	11,350	8,850	1.5 cm.
	B	12,341	4,920	3,599	8,906	6,726	
	C	77.5%	82%	59%	70%	76%	
7	A	16,950	11,750	9,600	14,300	14,050	1.4 cm.
	B	12,628	10,399	7,776	11,297	11,240	
	C	74.5%	88.5%	81%	79%	80%	
8	A	15,250	10,250	6,400	11,200	16,250	Regressed
	B	12,784	8,666	4,992	9,072	12,837	
	C	83.5%	84.5%	78%	81%	79%	
9	A	22,650	9,050	8,200	14,950	15,000	1.2 cm.
	B	19,026	6,878	5,412	10,913	10,350	
	C	84%	76%	66%	73%	69%	
10	A	22,550	3,800	Died	.....	.....	Dead
	B	18,266	.....	.....	.....	.....	
	C	81%	Anemic	3/21/34	.....	.....	
11	A	16,150	7,000	6,000	19,950	11,800	Regressed
	B	13,566	5,215	4,530	18,154	9,204	
	C	84%	74.5%	75.5%	91%	78%	
12	A	14,150	9,450	5,800	18,800	10,850	1.5 cm.
	B	12,892	7,655	4,611	16,434	7,920	
	C	88%	81%	79.5%	88%	73%	
Average number of lymphocytes	B	18,135	7,011	5,585	12,950	12,121	All but 3 grew progressively
	C	80.9%	78.9%	74.4%	82.2%	76.7%	

* A shows the total leukocyte count; B, the number of lymphocytes; C, the percentage of lymphocytes.

In the susceptible rats the growth of the cancer was not significantly altered by the decrease in the lymphocyte count. Eight of the eleven cancers (73 per cent) grew progressively as compared to eight of the nine in the susceptible control animals (90 per cent).

As in experiment 4 the susceptible rats again proved to be more responsive to the destructive action of the roentgen rays on the leukogenic structures as well as on the general health. As previously mentioned, the susceptible group was in such poor condition at the time of the seventh irradiation that the exposure was omitted. In spite of

the fact that the susceptible rats received 50 r less than the immune animals in the former the average lymphocyte count dropped from 18,135 to 5,585 (a difference of 12,550) as compared to the drop from 12,133 to 5,410 (a difference of 6,723) which occurred in the immune rats.

Microscopic examination of the tumors and surrounding tissues revealed the results listed in table 7.

Probably the most significant observation revealed in table 7 is the great decrease of lymphocytic infiltration and fibroblastic proliferation around the tumors in the immune rats receiving the roentgen rays.

TABLE 7.—*Effect of Irradiation on the Histologic Appearance of the Tumor and Surrounding Tissue*

Animal	Peritumoral Lymphocytic Infiltration	Peritumoral and Intratumoral Fibrosis	Tumor Tissue	Fate of Tumor
Immune, irradiated (rat 1).....	Slight	Slight	Viable	Regressed in 3 wks.
Immune, irradiated (rat 2).....	Slight	Rather slight	Viable	Regressed in 3 wks.
Immune, control (rat 1).....	Profuse	Moderate	Necrotic	Regressed in 3 wks.
Immune, control (rat 2).....	Profuse	Profuse, but poor stroma reaction	Viable at periphery only	Regressed in 3 wks.
Susceptible, irradiated (rat 1)..	Slight	Moderate	Viable	0.9 cm. at 3 wks.
Susceptible, irradiated (rat 2)..	Slight	Slight	Viable	1.7 cm. at 3 wks.
Susceptible, control (rat 1).....	Slight	Moderate	Viable	1.4 cm. at 3 wks.
Susceptible, control (rat 2).....	Slight	Profuse	Viable	1.1 cm. at 3 wks.

This is in contrast to the large amount of lymphocytic and fibrotic reaction seen in the immune rats not exposed to roentgen rays. Moreover, in the immune rats receiving roentgen rays the tumors appeared more viable than in the immune rats not receiving irradiation. From these observations it may be concluded that the lymphocytopenia resulting from the irradiation of the immune rats had prevented the lymphocytic infiltration and subsequent fibrosis around the tumor allowing it to grow unhampered by these defenses. The fact that the tumors promptly regressed, however, makes it appear probable that other defense mechanisms are also in force. Unfortunately the number of animals studied was too small to allow safe generalization, but the observations, so far as they go, are convincing because of the marked difference in every case between the tumors of the irradiated and of the control rats.

As would be expected, the difference between the irradiated and the control susceptible animals was less marked because all the animals were highly susceptible whether irradiated or not.

SUMMARY AND CONCLUSIONS

The marked lymphopenia produced by repeated roentgen irradiation did not destroy the immunity in a strain of cancer-resistant rats developed in this laboratory, nor did it alter the response of rats of a strain susceptible to cancer implants.

The pronounced lymphocytic and fibrotic response to cancer implants in immune rats was reduced in animals rendered lymphopenic by roentgen irradiation, but this did not keep the tumors from regressing promptly, so obviously other mechanisms of resistance are also operative.

Not only were rats of the cancer-susceptible strain more subject to the deleterious effects of roentgen rays on the supply of leukocytes than rats of the immune strain, but the general well-being of their bodies was also more disturbed.

Small doses of roentgen rays over a long period resulted in a degree of selective destruction of lymphocytes. Large doses reduced all the leukocytes with no evident selective action on any one type.

The part played by lymphocytes does not appear to be an important factor in maintaining the immunity to carcinoma so characteristic of our cancer-resistant strain of rats.

TUMOR METASTASIS

VI. OVARIAN METASTASIS OF CARCINOMA

SHIELDS WARREN, M.D.
AND
W. BRANDON MACOMBER, M.D.
BOSTON

Although the Krukenberg tumors of the ovary have received much attention, other tumors metastatic in the ovary have been somewhat neglected. In most of the studies little mention is made of the functional condition of the ovary or the relation to the menopause.

Primary cancers of the gastro-intestinal tract and, to a lesser extent, of the breast predominate. Stone [1] summarized from the literature 133 cases of secondary ovarian cancer, 102 of which were primary in the stomach and intestines. Ley [2] reported 38 ovarian metastases in 324 autopsies, one of the highest frequencies recorded. Of 50 malignant tumors of the ovary Bell [3] found 16 to be secondary; half of these were metastases from the gastro-intestinal tract. In 1928 Gauthier-Villars [4] collected reports of 355 cases of ovarian metastasis from carcinomas of the alimentary tract, 247 of which were of gastric origin. In a series of 37 metastatic tumors of the ovary Scarpitti [5] found only 2 originating from cancer of the breast. Paget [6] found ovarian metastases in 5 per cent of 735 cancers of the breast.

The route of ovarian metastasis has been varyingly ascribed to the blood stream, the lymph stream and transperitoneal implantation. The frequency with which the ovaries are involved in younger women whose ovaries have a better supply of blood and lymph, as compared with

---

From the Laboratories of Pathology of the New England Deaconess, Pondville, and Collis P. Huntington Memorial Hospitals.

The previous articles of this series are as follows: I. Distribution of Metastases in Carcinoma of the Cervix Uteri, Surg., Gynec. & Obst. **56**:742, 1933; II. The Distribution of Metastases in Cancer of the Breast, ibid. **57**:81, 1933; III. The Distribution of Metastases in Carcinoma of the Large Intestine, New England J. Med. **209**:167, 1933; IV. Metastases of Cancer of the Stomach, ibid. **209**:825, 1933; V. The Metastases of Carcinoma to the Spleen, Am. J. Cancer **21**:517, 1934.

1. Stone, W. S.: Surg., Gynec. & Obst. **22**:407, 1916.
2. Ley, G.: Proc. Roy. Soc. Med. **13**:95, 1919.
3. Bell, W. B., and Datnow, M. M.: Am. J. Cancer **16**:1, 1932.
4. Gauthier-Villars, P.: Contribution à l'étude des métastases des epithéliomas digestifs, Thèse de Paris, 1927; Prat. méd. franç. **9**:228, 1928.
5. Scarpitti, C.: Tumori **7**:47, 1933.
6. Paget, S.: Lancet **1**:571, 1889.

TABLE 1.—*Ovarian Metastases of Carcinoma*

Primary Site	Type of Carcinoma	Age	Duration		Ovarian Involvement		Probable Mode of Metastasis	
			Onset to Treatment	Total Duration	Right	Left	Vascular or Lymphatic	Transperitoneal
Right breast	Carcinoma simplex	50	?	2½ yrs.	+	+	+	..
Left breast	Carcinoma simplex	42	?	2 yrs.	+	0	+	..
Right breast	Adenocarcinoma	64	1 mo.	7 yrs.	+	+	+	..
Left breast	Carcinoma simplex	68	?	1 yr.	+	+	+	..
Right breast	Adenocarcinoma	42	?	6 mos.	+	+	+	..
Right breast	Carcinoma simplex	50	3½ yrs.	4 yrs.	+	+	+	..
Left breast	Adenocarcinoma	46	6 mos.	3 yrs.	+	0	+	..
Left breast	Carcinoma simplex	38	?	1 yr.	+	+	+	..
Right and left breast	Adenocarcinoma	39	?	1 yr.	+	+	..	+
Left breast	Carcinoma simplex	57	3 yrs.	3 yrs., 10 mos.	+	+	+	..
Right breast	Carcinoma simplex scirrhous	43	4 mos.	1 yr., 4 mos.	+	+	+	..
Left breast	Carcinoma simplex	63	5 mos.	6 mos.	+	0	+	..
Left breast	Carcinoma simplex	46	4 mos.	4 yrs., 4 mos.	+	+	+	..
Right breast	Carcinoma simplex	52	? few mos.	3 yrs.	+	+	..	+
Left breast	Carcinoma simplex	51	?	2 yrs.	+	+	+	..
Right breast	Carcinoma simplex	40	2 mos.	1 yr.	+	+	+	..
Stomach	Adenocarcinoma	54	?	8 mos.	0	+	+	..
Rectum	Colloid carcinoma	71	?	? 6 mos.	+	+	+	..
Stomach	Adenocarcinoma	27	?	2 yrs.	+	+	+	..
Stomach	Adenocarcinoma	52	No treatment	6 mos.	0	+	+	..
Ascending colon	Adenocarcinoma	71	8 mos.	1 yr.	+	+	..	+
Stomach	Adenocarcinoma	62	2 mos.	3 mos.	0	+	+	..
Stomach	Carcinoma simplex	51	1 mo.	2 mos.	+	+	+	..
Stomach	Carcinoma simplex	33	? few weeks	Still living	0	+	+	..
Stomach	Carcinoma simplex	47	6 mos.	6 mos., 1 week	+	0	+	..
Stomach	Adenocarcinoma	49	No treatment	6 mos.	0	+	+	..
Stomach	Carcinoma simplex	27	No treatment	1 yr., 8 mos.	+	+	+	..
Sigmoid	Malignant adenoma	51	3 mos.	4 mos.	+	0	+	..
Descending colon	Adenocarcinoma	56	? 2 yrs.	4 yrs.	0	+	..	+
Rectum	Adenocarcinoma	61	4 mos.	16 mos.	0	+	..	+
Rectum	Adenocarcinoma	60	2 yrs.	3 yrs.	+	+	+	..
Vulva	Epidermoid carcinoma grade II	69	?	4 yrs.	0	+	..	+
Uterus	Adeno-acanthoma	59	No treatment	1½ yrs.	0	+	..	+
Uterus	Adenocarcinoma	64	2 wks.	6 wks.	+	+	+	..
Cervix	Epidermoid carcinoma grade II	66	No treatment	2½ yrs.	0	+	..	+
Cervix	Epidermoid carcinoma grade II	51	3 yrs.	3-6 mos.	+	+	..	..
Uterus	Carcinoma simplex	72	4 mos.	8 mos.	+	+	..	+
Bladder	Epidermoid carcinoma grade II	56	10 mos.	1 yr.	+	0	+	+
Suprarenal gland	Adenocarcinoma	44	? 5 mos.	8 mos.	+	+	..	+
Primary not found	Carcinoma simplex	50	3 yrs.	3 yrs., 5 mos.	+	+	+	..
	Metastatic amelanotic melanotic sarcoma	30	1 mo.	3 yrs.	0	+	+	..

older women, leads one to expect these routes to be of major importance. However, transperitoneal metastasis is undoubtedly an important factor. One modification of the latter route has been emphasized by Sampson,[7] namely the transport through the tube and then transperitoneally to the ovary of fragments of carcinoma of the uterus. He also emphasized the

TABLE 2.—*Ovarian Metastases of Tumors of the Breast in Relation to Ovarian Function*

Age	Menstrual Function	Ovarian Tissue
50	—	Atrophic
42	?	Functioning
64	—	Atrophic
68	—	Atrophic
42	?	Functioning
50	—	Atrophic (cystic)
46	—	Atrophic
38	?	Destroyed
39	?	Destroyed
57	—	Atrophic
43	+	Functioning
63	—	Atrophic
46	+ (up to roentgen treatment)	Destroyed
52	—	Destroyed
51	—	Destroyed
49	+	Slight amount of functioning tissue

TABLE 3.—*Ovarian Metastases of Gastro-Intestinal Tumors in Relation to Ovarian Function*

Age	Menstrual Function	Ovarian Tissue
54	—	Atrophic
71	—	Atrophic and destroyed
27	+	Functioning
52	—	Atrophic
71	—	Atrophic
62	—	Atrophic
51	—	Destroyed
33	+	Functioning
47	+	Functioning
49	—	Functioning
27	+	Functioning
51	—	Atrophic
56	—	Atrophic
61	—	Atrophic
60	—	Atrophic

importance of the salpingeal lymphatics. Since, in the absence of generalized peritoneal implants, metastases to the ovary occur more commonly in the substance of the organ than on the peritoneal coat, the importance of the transperitoneal route is probably slight in other than intraperitoneal or uterine tumors.

In about 800 autopsies on women with malignant disease in the New England Deaconess, New England Baptist, Pondville and Collis P. Huntington Hospitals, we found 40 cases of secondary ovarian tumors. One surgical case is added. We have excluded cases in which the cap-

7. Sampson, J. A.: Surg., Gynec. & Obst. **38**:287, 1924.

sule of the ovaries showed implants as a part of a generalized peritoneal carcinosis. These observations are summarized in table 1.

The relative age of these patients is of interest, only 13 cases, or 33 per cent, occurring in the seventh, eighth and ninth decades. This contrasts with 46 per cent of cases in the same age group in a series of 1,059 autopsies on patients with malignant disease in the same hospitals. This tendency toward occurrence in the younger patients is marked in the case of the cancers of the breast. Twelve and a half per cent of the ovarian metastases of cancers of the breast occurred under the age of 40, although 10 per cent of the cancers of the breast occurred under this age. Likewise, 50 per cent occurred before the age of 50, although only 33 per cent of the cases of cancer of the breast occurred before that age. Thus, ovarian metastases are proportionately more frequent before than after the menopause (table 3).

In determining the frequency of ovarian metastases in our series we have ruled out all instances of primary ovarian tumors, even though one ovary might have appeared to be the primary site and the other to be involved by metastasis. In our experience cancer of the ovary is so frequently bilateral that we believe it unsafe to regard any case of ovarian involvement as a metastasis from the other ovary. Similarly, we have ruled out the great majority of the cases of cancer of the cervix uteri as in these cases widespread pelvic infiltration is very frequent and it is difficult to distinguish between extension and metastasis. We believed that if any error is to be made it is safer to understate the frequency and importance of ovarian metastases rather than to overemphasize them. Therefore, we have omitted all the cases of cervical cancer with ovarian involvement unless the discontinuity of the process could be clearly demonstrated. This accounts for the relatively small number of cervical cancers recorded in table 1.

Metastasis is much more frequently bilateral than unilateral, 25 as compared to 16 cases.

The duration of the disease is relatively short in the cases of ovarian metastasis, 23 patients showing a total duration of a year or under from the onset of symptoms to death, while only 4 lived over three years. In view of the frequency with which widely distributed metastases occur, particularly in cases of cancer of the breast, in the longer survival period, the rarity of long time survivals among the patients with ovarian involvement is of interest.

The two most frequent primary sites from which cancer metastasizes to the ovaries are the gastro-intestinal tract (15 cases) and the breast (16 cases). These two primary sites represent 76 per cent of our cases. The frequency of metastasis of carcinoma of the breast to the ovaries is even greater in comparison with metastases of gastro-intestinal tumors than appears from these figures, as the series contains approximately 3

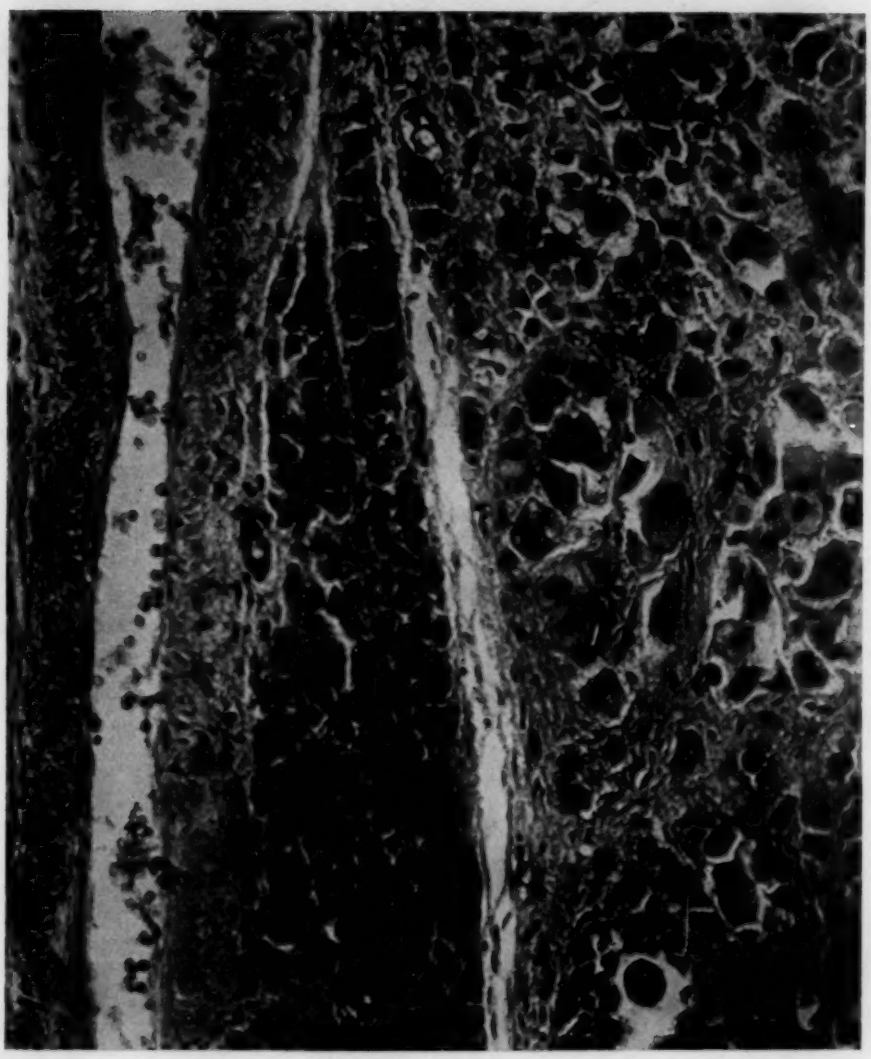


Fig. 1.—Metastasis to the ovary of carcinoma of the suprarenal medulla. Margin of a large intra-ovarian nodule (× 300).

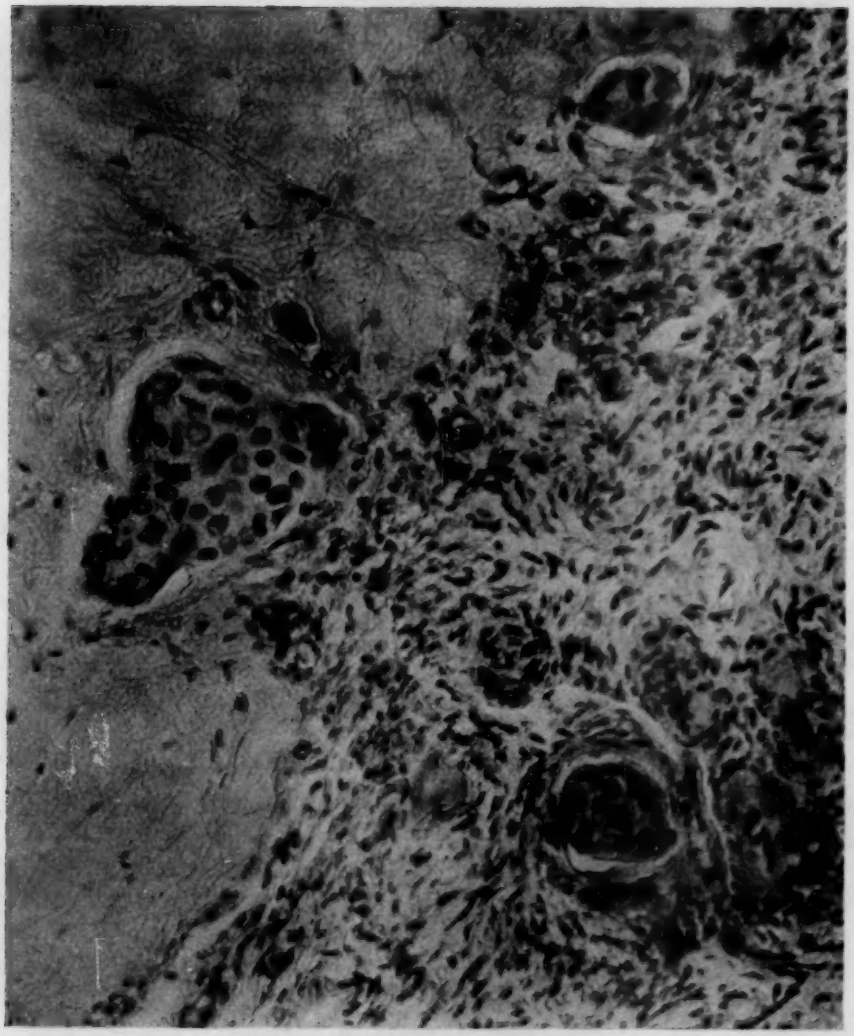


Fig. 2.—Intra-ovarian metastasis of carcinoma simplex of the breast. Extensive intravascular growth and numerous foci of carcinoma in corpora albicantia (× 300).

cases of gastro-intestinal cancer for every 2 cases of cancer of the breast. Since the material of three hospitals doing extensive work in malignant disease has been drawn on for this study, a fairly representative sample of the forms of malignancy encountered in the general population has been obtained, and the autopsy material is not unduly weighted with any one form of the disease.

TABLE 4.—*Relation of Ovarian to Other Metastases*

Primary Tumor	Age	Extent of Metastasis
Carcinoma simplex of right breast	50	Distant (very extensive)
Carcinoma simplex of left breast	42	Distant (very extensive)
Adenocarcinoma of right breast	64	Distant (very extensive)
Carcinoma simplex of left breast	68	Distant (very extensive), both tubes also involved
Adenocarcinoma of right breast	42	Distant
Carcinoma simplex of right breast	50	Distant (very extensive)
Adenocarcinoma of left breast	46	Distant (very extensive)
Carcinoma simplex of left breast	38	Distant (very extensive)
Adenocarcinoma of right breast	39	Distant (very extensive)
Carcinoma simplex of left breast	57	Distant (very extensive)
Carcinoma simplex (scirrhous) of right breast	43	Distant (very extensive)
Carcinoma simplex of left breast	63	Distant (very extensive), uterus and broad ligament involved
Carcinoma simplex of left breast	46	Distant (very extensive), uterus also involved
Carcinoma simplex of right breast	52	Distant (very extensive), cervix and vagina also involved
Carcinoma simplex of left breast	51	Distant (very extensive)
Carcinoma simplex of right breast	49	Distant (very extensive)
Adenocarcinoma of stomach	54	Distant
Colloid carcinoma of rectum	71	Distant, uterus also involved
Adenocarcinoma of stomach	27	Regional nodes, only ovaries
Adenocarcinoma of stomach	52	Distant
Adenocarcinoma of ascending colon	71	Distant
Adenocarcinoma of stomach	62	Distant
Carcinoma simplex of stomach	51	Distant, uterus also involved
Carcinoma simplex of stomach	33	Regional nodes, only ovaries involved
Carcinoma simplex of stomach	47	Regional nodes, right ovary
Adenocarcinoma of stomach	49	Distant
Carcinoma simplex of stomach	27	Distant, uterus and cervix involved
Malignant adenoma of sigmoid	51	Right kidney, liver, appendix and right ovary
Adenocarcinoma of descending colon	56	Regional nodes, broad ligament and uterus also involved, with left ovary
Adenocarcinoma of rectum	61	Regional nodes, pelvic contents and bone
Adenocarcinoma of rectum	60	Distant, uterus also involved
Epidermoid carcinoma, grade II, of vulva	69	Regional nodes, pelvic contents
Adeno-acanthoma of uterus	59	Distant
Adenocarcinoma of uterus	64	Distant
Epidermoid carcinoma, grade II, of cervix	66	Distant
Epidermoid carcinoma, grade II, of cervix	51	Regional nodes, pelvic contents
Carcinoma simplex of uterus	72	Distant, both tubes also involved
Epidermoid carcinoma, grade II, of bladder	56	Regional nodes, pelvic contents
Adenocarcinoma of suprarenal gland	44	Distant, uterus also involved
Carcinoma simplex (primary not found)	50	Distant (abdomen only)
Metastatic amelanotic melanotic sarcoma	30	Distant

From table 3 it may be noted that in all the cases in which the cancer had occurred prior to the menopause some functioning ovarian tissue was present.

Other metastases are frequent when the ovary is involved; in only 5 cases was there restriction to the regional lymph nodes and the ovaries or to the ovaries alone. One of these patients is still alive, so the full metastatic extent is as yet unknown. When the patient was 33 years old a subtotal resection of the stomach was done for a primary carcinoma

of the stomach and a fairly extensive carcinoma simplex was removed. Three years later the patient reentered the hospital with pain in the lower part of the abdomen, and a mass was found in the pelvis. Both ovaries were resected and found to be the seat of typical Krukenberg tumors. At the present time the patient is living and well.

The relationship of the ovarian metastases to other metastases is shown in table 4.

The youth of several of the patients with cancer of the stomach is very striking, 2 dying at 27 and 1 still alive at 33. In only 3 of the cases of cancer of the breast with very wide metastases was there evidence of transperitoneal ovarian implantation, and in these cases there was evidence of transmission by vascular channels as well. Similarly, only 3 of the gastro-intestinal tumors showed evidence of transperitoneal metastasis. In several of the cases the extent of the tumor in the ovary had produced an artificial menopause.

SUMMARY AND CONCLUSIONS

Forty-one cases of ovarian metastases are reported. Metastases are disproportionately frequent in functioning ovaries. In this series, cancers of the breast metastasized to ovaries as frequently as did gastro-intestinal cancers. Ovarian metastases are usually associated with widespread metastatic involvement of other organs.

SCARLET RED AS A POSSIBLE CARCINOGENIC AGENT

AN EXPERIMENTAL STUDY

CLARENCE T. ECKERT, M.D.

ZOLA K. COOPER, PH.D.

AND

M. G. SEELIG, M.D.

ST. LOUIS

In 1906 Fischer,[1] who was interested in the effect of chronic irritation on the skin, showed that scarlet red in oil, when injected under pressure into the rabbit's ear, produced a marked epithelial proliferation similar to that found in carcinoma, though not to be classed as such. Since that time scarlet red has been widely used therapeutically as a tissue stimulant [2] in the healing of wounds, without any other effect than the production of epithelial hyperplasia. Many investigators, after making only one injection of scarlet red subcutaneously and then observing the effects on the skin for but a relatively short period of time, have reported contradictory results.

Coal tar, at present the most useful agent for the production of experimental cancer, was shown to be definitely carcinogenic only after Yamagiwa and Ishikawa [3] had used it continuously over a prolonged period. Fischer also realized in the instance of scarlet red the necessity of repeated applications over a long period of time (how long he did not state), but there is no record of the use of long-continued, combined surface and subepithelial applications of this dye. Our purpose in the present experiments was to determine whether or not scarlet red is carcinogenic when applied in this combined manner over an extended length of time. A careful consideration of the literature, which we discuss later, makes it plain that the problem of the possible carcinogenicity of scarlet red calls for a conclusive study.

From the Barnard Free Skin and Cancer Hospital.

This study has been aided by a grant from the Committee on Scientific Research of the American Medical Association.

1. Fischer, B.: München. med. Wchnschr. **53**:2041, 1906; Verhandl. d. deutsch. path. Gesellsch. **10**:20, 1906.

2. Haga gives an excellent bibliography on the therapeutic use of scarlet red as a stimulant of epithelial growth in the Zeitschrift für Krebsforschung (**12**:525, 1913).

3. Yamagiwa, K., and Ishikawa, T.: J. Cancer Research **3**:1, 1918.

EXPERIMENTS

Methods and Materials.—A series of 161 young white mice, from 2 to 3 months of age, of mixed breed from a general population was used. A 2.5 per cent solution of scarlet red in olive oil was both painted on the surface of the skin and injected subcutaneously. Olive oil was used as a medium in order to prolong the contact of the tissue and dye, and also because of its relative blandness compared with the mineral oils. The scarlet red was ground as finely as possible before compounding the preparation at room temperature.

The mice were painted three times a week for a total of one hundred and twenty-nine applications during a period of three hundred and twelve days over a site 3 cm. in diameter on the dorsal surface of the neck. At the beginning of the experiment sodium sulphide was used to depilate the area to be painted. However, since the regrowth of hair was very rapid (in from three to five days) depilation was soon discontinued because it was feared that such frequent irritation might prove to be an uncontrolled factor.

Over the same period of three hundred and twelve days, seven subcutaneous injections of from 0.50 to 0.75 cc. of the scarlet red oil were made as nearly as possible under the center of the painted area. Only seven injections of oil were made, because in every instance we waited until the oil from the previous injection seemed to be largely absorbed before injecting a fresh supply.

Observations.—The majority of the subcutaneous injections produced globular fluctuating swellings from 1 to 1.5 cm. in diameter which persisted for from five to six weeks. In some of the mice, however, either the swelling disappeared in from two to three weeks after the injection or the oil spread immediately beneath the skin without localizing. On postmortem examination the localizations of oil were determined to be due to encystment by a thin capsule of fibrous tissue. The only other observation of note at autopsy was the presence of scarlet red in the omental and mesenteric fat and occasionally in the fat of the anterior abdominal wall. This same observation was noted by Fischer. No collection of the dye was noted grossly in the lymph glands.

During the period of experimentation no gross changes were noted locally, and the animals at no time showed any signs of toxicity. The mortality was in fact about the same as that of the general population, with 64 of the original 161 animals surviving at the end of the experiment. We were unable at any time to demonstrate falling of hair from, or ulceration of, the sites of external application. Palpations of the latter at frequent intervals failed to reveal induration or roughening.

Tissue for microscopic study from the site of injection was taken at frequent intervals during the course of the experiment and, of course, specimens were obtained ultimately from all the animals. Each block included skin, subcutaneous tissue and muscle. The tissue was fixed in a solution of formaldehyde and stained with hematoxylin and eosin and also by Van Gieson's stain for connective tissue.

The most significant microscopic changes were those in the epithelial layer. Nothing was seen which could be interpreted as indicating a tendency to malignant change. Hyperplasia was found in only 18 animals, in 12 of which this change was very slight, in 3 moderate and in 3 marked. All of the latter occurred late in the experiment, after from two hundred and sixty-three to three hundred and twelve days. Hyperkeratosis was observed in 10 mice. In only 2 of them was the lesion pronounced; in one of these it occurred early in the experiment (after thirty-three days) and in the other late (after two hundred and sixty-three days). No marked variations were encountered on examination of the hair follicles, though in 5

animals from slight to moderate hyperplasia was observed. The sebaceous glands revealed no hyperplastic changes and possibly were fewer and smaller than is usually the case in normal skin of this region.

In 54 animals the sections showed a typical reaction of the foreign body type to the subcutaneous injection. Fibrosis was demonstrable in 54 mice; it was present in an advanced stage in 25, moderate in 9 and early in 20. The reaction was a progressive one. First a thin capsule of fibrous tissue localized the injected material, and then the latter was invaded by a fine network of fibrous strands which divided the mass into hundreds of smaller globules. The next step seemed to be a replacement of the oil and dye by a mixture of young fibrous tissue, small round cells, a few capillaries and a small amount of elastic tissue. The last stage showed a marked increase of fibrous and elastic tissue with the frequent occurrence of typical peripheral nucleated giant cells. In 7 instances this reaction was present in the muscles, evidently because the injection into these mice was intramuscular rather than subcutaneous.

We encountered no evidence, either grossly or microscopically, indicating malignancy. There was no suspiciously pronounced dipping or pegging of the epithelium or increase in mitoses in the basal epithelial layer, and the basement membrane was never found to be broken through. The few cases of hyperplasia and hyperkeratosis noted were, in our opinion, probably due to the irritation of the frequent external applications.

REVIEW OF THE LITERATURE

A review of the literature on the experimental use of scarlet red shows how contradictory and confusing have been the results of the many workers in this field.

Fischer [1] in 1906 was the first to inject subcutaneously, under pressure, a saturated solution of scarlet red in olive oil into the ears of rabbits. This produced in the connective tissue cellular infiltration, formation of giant cells and the production of a rich new growth of young connective tissue. In the epithelium Fischer observed an increase in the number of mitoses in the basal layer as well as in the sebaceous glands and hair follicles, marked keratosis and the production of epithelial downgrowths which surrounded the oil drops contained in the spaces of the connective tissue. The connective tissue finally became shot through with cords of epithelium producing a picture which Fischer considered comparable to that of squamous cell carcinoma in man. He attributed this epithelial reaction to the strong chemotactic effect of scarlet red in oil on the epithelium and called the active element in the dye an attraxin, but he specifically stated that the process could not be considered to indicate formation of cancer, and that the reaction of the epithelial cells ceased after the injected oil was absorbed. Fischer's articles leave the reader in some doubt, however, so far as he actually did say that the process was comparable to carcinoma, that he found the downgrowth of squamous cells invading a lymph space and, furthermore, that the proliferating epithelium, like that in cancer, had the shape of a branching tree.

Jores [4] repeated Fischer's work and obtained similar results. He believed that the proliferative process began in the cells of the hair follicles and that hypertrophy of the epithelium of the surface occurred only when injections were made with considerable force. He considered this proliferation to be essentially a regenerative one. Ritter [5] and Stoeber [6] (1909) also confirmed Fischer's results.

Helmholtz,[7] after injecting scarlet red oil into the skin of the rabbit's ear, obtained an epithelial proliferation similar to that which Fischer produced. He also injected scarlet red into the epithelium of the bladder, esophagus, stomach, rectum and mouth, but obtained epithelial downgrowths only in the mouth and rectum.

Marie and Clunet [8] injected scarlet red oil into 5 rabbits, and twenty to thirty days after injection found in 4 of the animals metaplasia in the hair follicles and sebaceous glands and voluminous cysts which contained keratin in the epidermis. In one rabbit a small tumor appeared which was made up of a stroma of connective tissue rich in cells and blood capillaries "in the middle of which grew in all directions epithelial arborescences enclosing typical epithelial pearls."

McConnell [9] injected into the external surface of the right ear of one Belgian hare about 4 minims (0.24 cc.) of a saturated solution of sudan III in olive oil. Nine days after the single injection he found "very long and complicated prolongations (of the epithelium) extending into the deeper structures," as well as isolated masses of epithelium "lying well down near the cartilage." McConnell felt that such injections might, if continued further, produce cancer. Stahr [10] obtained results quite similar to those of McConnell after a single injection of sudan III.

By the injection of scarlet red oil Seckel [11] obtained epithelial growths in rabbits' ears which grossly resembled squamous cell carcinoma, but which regressed with the gradual disappearance of the scarlet red oil.

Haga [2] found that the injection of the dye in oil caused proliferation not only in the skin but in the mucous membranes of the tongue and stomach, the endothelium of the lymph vessels and the epithelium of the bile duct and the excretory ducts of the mammary glands. He

4. Jores, L.: München. med. Wchnschr. **54**:879, 1907.

5. Ritter: München. med. Wchnschr. **54**:542, 1907.

6. Stoeber, H.: München. med. Wchnschr. **57**:739, 1910.

7. Helmholtz, H.: Bull. Johns Hopkins Hosp. **18**:365, 1907.

8. Marie, P., and Clunet, J.: Bull. Assoc. franç. p. l'étude du cancer **2**:146, 1909.

9. McConnell, G.: J. A. M. A. **49**:1498, 1907.

10. Stahr, H.: München. med. Wchnschr. **54**:1178, 1907.

11. Seckel: München. med. Wchnschr. **55**:199, 1908.

believed that these changes were due to chemical irritation of the scarlet red. No true carcinomas were obtained but the changes in the mucous membrane were thought to show "a definite tendency to malignancy."

Barratt [12] also confirmed Fischer's findings and made cytologic studies of the epithelial growths which he obtained, reporting the occurrence of numerous "reduced" mitoses in them.

The production of carcinoma in other organs than the skin through the use of scarlet red was mentioned by Eggers [13] in his recent review. He cited the work of Yamagiwa and Ohno,[14] who produced carcinomas in fowls by the injection of scarlet red oil into the fallopian tube.

Scarlet red has likewise been shown by Werner [15] to accelerate greatly the growth of inoculated tumors in mice.

All of these investigations more or less confirmed Fischer's conclusions. In addition some of these workers leaned toward the belief that scarlet red might have carcinogenic properties. Many other workers have failed to obtain similar results.

Snow [16] injected a sterile saturated solution of scarlet red in olive oil under the skin of the outer aspect of the ears of 9 rabbits, and found that although it was present in the tissues from seven to sixty-one days, the dye had no effect on the epithelial cells. It produced only a low grade inflammatory reaction. Geipel [17] likewise was unable to produce neoplastic changes by the injection of scarlet red oil into the ears of rabbits. He thought that the proliferation which did occur arose from epithelial cells which had been misplaced into the deeper tissue by the injection. Benthin [18] also called attention to the part played by the puncture of the needle in producing the epithelial hyperplasia and considered the effect from the points of view of reaction to inflammation, tension in tissues and possibly circulatory disturbance.

Levin [19] made subcutaneous injections into white rats of a saturated solution of scarlet red in soft paraffin. Three weeks later the paraffin with the tissue surrounding it was excised and studied microscopically. Only a connective tissue reaction was found. Five subcutaneous injections of scarlet red in oil at intervals of forty-eight hours likewise affected only the connective tissue.

---

12. Barratt, J.: Proc. Roy. Soc., London, s. B **79**:372, 1907.

13. Eggers, H. E.: Arch. Path. (*a*) **12**:983, 1931; (*b*) **13**:112, 1932; (*c*) **13**: 296, 1932; (*d*) **13**:462, 1932.

14. Yamagiwa, K., and Ohno, S.: Gann **12**:3, 1918; quoted by Eggers.[13b]

15. Werner, R.: München. med. Wchnschr. **55**:2267, 1908.

16. Snow, C.: J. Infect. Dis. **4**:385, 1907.

17. Geipel: München. med. Wchnschr. **54**:1057, 1907.

18. Benthin, W.: Ztschr. f. Krebsforsch. **10**:227, 1910.

19. Levin, I.: J. Exper. Med. **10**:811, 1908.

Stoeber [6] and Wessely [20] injected scarlet red oil into human skin but produced only epithelial hyperplasia.

Huguenin [21] and von Hansemann [22] attempted to transplant the epithelial growths obtained in rabbits' ears after injection of scarlet red, but were unable to do so. They consequently concluded that there was no tendency to formation of malignant tumors as a result of the injection of the dye.

Bullock and Rhodenburg,[23] working on large numbers of animals, 178 rabbits, 89 rats and 122 mice, found that the reaction caused by the injection of scarlet red in oil is a reparative process "dependent for its inception and continuation upon irritation and cell death." They obtained a certain degree of metaplasia in the tissues of the ear, breast, prostate and lung following injections of the dye, but no true malignant changes.

Mori [24] in rabbits and Burckhardt [25] in rats produced artificial cysts in the skin by inverting flaps of skin and suturing the skin together over them. They then injected scarlet red oil into these cysts. Epithelial proliferation occurred but no carcinomatous growth. Mori believed that this increased epithelial growth originated in the hair follicles. When his experiments were performed on cocks' wattles, which contain no cutaneous appendages, no atypical changes were found.

A number of workers have attributed the action of scarlet red to its effects on the circulation. Wyss [26] considered the epithelial proliferation produced to be a mechanical process due to an interference with the blood supply. Greischer and Schmincke [27] induced an artificial anemia and hyperemia in the ears of rabbits and found that this procedure did not alter the results of injection. Meyer,[28] however, showed that decrease in the blood supply accelerated the action of the scarlet red.

Attempts have been made to combine scarlet red with other substances in order to increase its effectiveness. Puccinelli [29] placed blocks

---

20. Wessely, K.: Med. Klin. **6**:542, 1910.
21. Huguenin, B.: Arch. de méd. expér. et d'anat. path. **22**:422, 1910.
22. von Hansemann, D.: Ztschr. f. Krebsforsch. **12**:398, 1913.
23. Bullock, F. D., and Rhodenburg, G. L.: J. M. Research **33**:53, 1915.
24. Mori, T.: Virchows Arch. f. path. Anat. **208**:333, 1912.
25. Burckhardt, H.: München. med. Wchnschr. **72**:1237, 1925.
26. Wyss, O.: München. med. Wchnschr. **54**:1576, 1907.
27. Greischer, L., and Schmincke, A.: München. med. Wchnschr. **58**:1607, 1911.
28. Meyer, A. W.: Beitr. z. path. Anat. u. z. allg. Path. **46**:437, 1909.
29. Puccinelli, E.: Pathologica **23**:73, 1931; abstr., Am. J. Cancer **15**:2836, 1931.

containing equal parts of tar, paraffin and scarlet red in the bladders of 26 rats and found that diffuse papillomas appeared in 8 animals. No metastases occurred, however, and the tumors were not histologically malignant. This work contradicted that of Maisin and Picard,[30] who believed that they produced malignant growths by this method.

Eber, Klinge and Wacker [31] fed mice on a diet rich in scarlet red and cholesterol and found that they could thus stimulate the development of experimental tumors produced by tarring these mice.

Fischer-Wasels [32] administered arsenic to produce a constitutional susceptibility before and during the time that he made injections of scarlet red into the breasts of rats. He reported the production of true carcinomas by this method.

Strauch and Bernhardt [33] found that splenectomy or other interference with the reticulo-endothelial system did not increase the possibility of obtaining malignant changes with scarlet red.

Dixon [34] obtained a simple epithelial hyperplasia in rabbits by the use of either scarlet red or sudan III, but produced no neoplastic changes. He also found that roentgen irradiation inhibited the epithelial proliferation produced by sudan III.

COMMENT

Our failure to obtain any marked degree of epithelial hyperactivity leads to speculation when contrasted with the carcinoma-like lesions produced by Fischer,[1] Jores,[4] Ritter [5] and others. It is possible that the growths noted by the latter were due to a reaction to a physical change, from the forced pressure with which the oil was injected, easily produced in a part like the ear of a rabbit, in which the skin and cartilage are in close and firm anatomic relation. In fact Jores [4] pointed out that he obtained the epithelial proliferation only when the injections were made with considerable force. Conversely the lack of sufficient pressure may have been instrumental in the failure of others to obtain hyperplasia from injection into the ear of the rabbit. The explanation of Geipel [17] and of Benthin [18] that the injecting needle may displace epithelial cells deeper into the tissues is possible though improbable. Certainly in our relatively large series, in which there were ample opportunities for this to take place, we found no evidence of this accident.

30. Maisin, J., and Picard, E.: Compt. rend. Soc. de biol. **96**:1058, 1927.

31. Eber, W.; Klinge, F., and Wacker, L.: Ztschr. f. Krebsforsch. **22**:359, 1925.

32. Fischer-Wasels, B.: München. med. Wchnschr. **75**:73, 1928.

33. Strauch, C. B., and Bernhardt, H.: Ztschr. f. Krebsforsch. **26**:370, 1927.

34. Dixon, R. L.: J. Infect. Dis. **6**:205, 1909.

While a physical change, such as increased pressure, might also account for the tumors produced by scarlet red in such tense tissues as the breast, prostate, ear and lung, it certainly would not account for the growths produced in such relatively lax structures as the rectum and fallopian tubes. This, then, would indicate an irritating or stimulating activity of the dye, which we were unable to demonstrate to any degree.

Of interest in our series was the presence of the dye, as also described by Fischer, in the fat of the anterior abdominal wall, omentum and mesentery, none of which showed any gross evidence of formation of tumor tissue. Microscopic sections of several blocks of the omentum revealed no histologic changes. The scarlet red probably reached these parts by direct extension, though this is conjectural.

CONCLUSION

Scarlet red, in a 2.5 per cent solution in olive oil, when applied simultaneously to the skin and subepithelial tissue of a given area (dorsal cervical region) of the skin of a mouse over a prolonged period of time is not carcinogenic. The dye as used is not toxic.

Case Reports, Laboratory Methods and Technical Notes

GENERALIZED OSTEOSCLEROSIS WITH CHRONIC POLYCYTHEMIA VERA

EDWIN F. HIRSCH, CHICAGO

The essential polycythemia (vera or rubra) is characterized [1] by a persistent increase of the erythrocytes of the blood (the number being usually between ten and fourteen million per cubic millimeter), by a plethoric appearance of the patient and by a marked reddening of the face and mucous membranes. The spleen is enlarged, and the volume of the blood is increased. The marrow tissues of the bones regularly are dark red or even black-red, a coloration which exceeds that in chronic anemia and in other hyperplasias of the hematopoietic tissues. The marked hyperplasia of the bone marrow is manifested further by an extension of the myeloid tissues into parts of the marrow usually fatty. Microscopically, there is a marked overgrowth of myeloid cells, among which are many megakaryocytes. Extramedullary hyperplasia of myeloid tissue occurs mainly in the spleen, liver and lymph nodes, and occurs in other viscera also.

Ideas of the nature of the disease postulate some stimulus which provokes a hyperplasia of the myeloid tissues. The amount of red cell disintegration seems not to be decreased; on the contrary, evidence of greater destruction is usually demonstrable. The hyperactivity of the marrow tissues is manifested clinically by the presence of both abnormal and immature red blood cells in the peripheral circulation. Accordingly, the disease in some patients is manifested in changes in the peripheral blood comparable to those in pernicious anemia.

In the published accounts of the disease, mention is made of the hyperplasia of the marrow, but not of a generalized osteosclerosis of the bones. Changes of the latter description were found during the

Aided by the Winfield Peck Memorial Fund.

From the Henry Baird Favill Laboratory of St. Luke's Hospital, Chicago, and the Norman Bridge Pathological Laboratory of Rush Medical College of the University of Chicago.

1. Minot, George R., and Buckman, Thomas E.: Am. J. M. Sc. **166**:469, 1923. Askanazy, Max: Morbus Vaquez, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1927, vol. 1, pt. 2, p. 942. Naegeli, Otto: Polycythemia vara, Blutkrankheiten und Blutdiagnostik, ed. 5, Berlin, Julius Springer, 1931, p. 568. Zadek, I.: Deutsche med. Wchnschr. **52**:1466, 1926. Gülke, Hans: Folia haemat. **38**:396, 1929.

postmortem examination of the body of a patient whose polycythemia was known to have existed for thirty-one years. Dr. C. L. Dougherty gave me permission to use this material and kindly obtained the clinical record for me.

REPORT OF CASE

On May 13, 1910, G. F. H., a white salesman, aged 32 years, entered the Presbyterian Hospital, in the service of Dr. B. W. Sippy, because of an enlarged spleen, blurred vision, nausea and vomiting, constipation and a voracious appetite. Enlargement and hardness of the left side of the abdomen had been noted in 1902. Nausea and vomiting began then. The patient in 1910 was engaged in strenuous outdoor work. The spleen was 1 handbreadth and the liver 3 fingerbreadths below the costal arch; the systolic blood pressure was 130 mm. of mercury; the erythrocytes were 11,350,000, the leukocytes, 12,050, per cubic millimeter, and the hemoglobin was 118 per cent (Dare). The urine contained a small amount of albumin. The disorder was diagnosed polycythemia vera. A right inguinal hernia was corrected surgically on March 12, 1914, by Dr. Dean Lewis, and a left on June 6, 1914.

On March 11, 1914, examination of the blood showed: erythrocytes, 6,980,000; leukocytes, 18,100; hemoglobin, 90 per cent (Tallqvist); coagulation time, three and one-half minutes. A differential count (Wright's stain) disclosed among erythrocytes, anisocytosis, poikilocytosis and 3 normoblasts; among leukocytes (250), 0.8 per cent small lymphocytes, 4.8 per cent large lymphocytes, 8.8 per cent transitional cells, 80 per cent polymorphonuclear neutrophil leukocytes, 2.8 per cent myelocytes (mononuclear neutrophils), 0.8 per cent myelocytes (mononuclear eosinophils) and 2 per cent eosinophil polymorphonuclear leukocytes.

On June 26, 1920, examination of the blood showed: erythrocytes, 5,525,000; leukocytes, 8,800; hemoglobin, 80 per cent. There were anisocytosis and poikilocytosis of the red cells. The leukocytes comprised small lymphocytes, 10 per cent; large lymphocytes, 20 per cent; polymorphonuclear neutrophil leukocytes, 68 per cent, and polymorphonuclear eosinophil leukocytes, 2 per cent.

A note of the physical condition on Aug. 25, 1920 (patient aged 43 years), stated that the spleen extended 1 fingerbreadth above the level of the umbilicus.

On Dec. 27, 1920, the blood count revealed 5,680,000 erythrocytes, 13,000 leukocytes and 90 per cent hemoglobin; on July 3, 1924, 6,260,000 erythrocytes, 15,700 leukocytes and 96 per cent hemoglobin; on June 30, 1931, 5,570,000 erythrocytes, 14,100 leukocytes and 78 per cent hemoglobin. On the latter date there were marked polychromatophilia, anisocytosis and poikilocytosis of the erythrocytes. The leukocyte count was: large mononuclear cells, 15 per cent; small mononuclear cells, 6 per cent; neutrophil leukocytes, 69 per cent; eosinophil leukocytes, 1 per cent; basophil leukocytes, 6 per cent and abnormal leukocytes, 3 per cent.

In April 1932, the erythrocyte count was 7,430,000.

On Oct. 22, 1933, the patient, then aged 55 years, entered St. Luke's Hospital because of urinary obstruction for two weeks. He had had frequency, nocturia and urgency for many years. No treatment had been prescribed for the polycythemia except roentgen therapy in 1923 for the enlarged spleen. The physical examination demonstrated, in addition to the conditions already mentioned, a markedly enlarged prostate. The erythrocyte count ranged between 4,600,000 and 5,300,000; the percentage of hemoglobin between 65 and 68, and the leukocyte count between 24,000 and 28,750. There were anisocytosis and poikilocytosis of the erythrocytes and

the presence of normoblasts and megaloblasts and of some immature myelogenous leukocytes. The bleeding time was two minutes and fifty seconds, and the coagulation time was four minutes. A transurethral electroresection of the prostate on Nov. 3, 1933, was followed by a marked hemorrhage into the urinary bladder. Death occurred twenty hours after the operation and about thirty-one years after polycythemia vera had been diagnosed.

Autopsy.—The essentials of the anatomic diagnosis based on the routine postmortem examination of the thorax and abdomen were: polycythemia vera; recent partial transurethral electroresection of the prostate gland; huge recent hemorrhage into the urinary bladder; bilateral retrograde hemo-ureter and hemopelvis of the kidney; anemia; splenomegalia and hepatomegalia (chronic myeloid hyperplasia); hyperplasia of the marrow and marked osteosclerosis of the bodies of the vertebrae, ribs and right femur, and glandular hyperplasia of the prostate.

The body weighed 159 pounds (72.1 Kg.) and was 162 cm. long. The skin was slightly jaundiced. The right and left axillary lymph nodes were small. The urinary bladder extended 12 cm. above the symphysis pubis. The symmetrically enlarged spleen reached 18 cm. below the left costal margin, was 30 cm. long, 22 cm. wide and 14 cm. thick. It was not adherent to adjacent viscera. Several red-brown lymph nodes in the fat tissues of the thymic body were from 1.5 to 2 cm. in diameter. More were about the large vessels arising from the aorta, and still others were in the hilum of the left lung. The periaortic abdominal lymph nodes were gray-red and from 1.5 to 2 cm. in diameter. The fluid and clotted blood recovered from the urinary bladder weighed 400 Gm. The entire circumference (9 cm.) of the neck of the urinary bladder from the verumontanum to 7 cm. behind was charred and grooved by the electric cautery loop. The prostatic tissues remaining on the left side measured 4 by 3 by 2.5 cm., and those on the right side 4 by 2.5 by 2 cm. There was a marked glandular hyperplasia of these tissues. The lining of the urinary bladder was hyperemic and had had many recent hemorrhages. The external diameter of the right ureter was 2.5 cm. The pelvis of the right kidney and the lumen of the right ureter were filled with fluid and clotted blood. The pelvis of the left kidney and the left ureter contained a small quantity of blood. There were no marked changes of the kidneys. The liver weighed 3,220 Gm. The capsule was slightly thickened by fibrous tissue and focally depressed. The edges were rounded and the hepatic tissues were firm. The surfaces made by cutting were tan-yellow with prominent central veins and lighter peripheral lobular tissues. There were marked cloudy swelling and slight fatty changes. The spleen weighed 3,400 Gm. The capsule generally was thickened by fibrous tissue and further had small scattered sclerotic thickenings. The edges were rounded. The broad surfaces made by cutting presented dark purple-red soft splenic tissue mottled by nodules of red-brown tissue from 4 mm. to 1 cm. in diameter. The tissues around these were dark red. There were scattered calcium plaques, some in the walls of blood vessels, others apparently in the splenic tissues.

The left and right fourth and fifth ribs, the bodies of the second, third, fourth and fifth lumbar vertebrae, and the shaft of the right femur were examined. There was a marked increase in the density of the rib tissues (fig. 1)—an extension of the compacta into the spongiosa so that the latter was reduced to a small amount. The other bones also were markedly sclerosed. In the bodies of the lumbar vertebrae (fig. 2) the tan-red marrow was reduced to approximately one fifth of the usual volume.

Histology.—The routine histologic examinations of the trachea, seminal vesicles, gallbladder, epididymis, pancreas, skeletal muscle, aorta, stomach, duodenum, urinary bladder, myocardium and testis disclosed no unusual changes. There were a few hyaline glomeruli in the renal tissues, and in the prostate marked glandular hyperplasia, necrosis, edema and suppurative inflammation.

The spleen had marked diffuse and also focal hyperplasias of myeloid tissue. The focal aggregations corresponded to the nodules of gray-brown tissues noted grossly on the surfaces made by cutting. These nodules in the spleen were the portions optimum for the histologic study of the myeloid elements. They contained aggregates of megakaryocytes and cells of the leukoblastic and erythroblastic series (fig. 3 *A*). There were also recent and old hemorrhages associated with many large phagocytes containing brown granular blood pigment. Continuous with trabeculae, mainly, were hyaline fibrous scars. These fibrous tissues contained masses of granular brown blood pigment and iron-incrusted fibers. The myeloid tissue infiltrations of the liver were small aggregates of immature cells and a few megakaryocytes. The lungs had the changes of chronic passive hyperemia. The alveolar walls were thick, and the alveolar spaces contained aggregates of large mononuclear phagocytes with brown blood pigment and recently extravasated erythrocytes. Perivascular and peribronchial fibrous scars (fig. 3 *B*) had iron-incrusted fibers [2] and brown granular material. About some of these incrusted fibers were foreign body giant cells, and certain scar tissues included also small masses of myeloid tissue.

The thymic, parabronchial and abdominal lymph nodes had the usual basic tissue pattern. There was a hyperplasia of the mononuclear phagocytes in the sinusoids, and focally along the sinusoids were small masses of myeloid tissue with megakaryocytes and myeloid cells not clearly differentiated.

The bodies of the lumbar vertebrae, the ribs and the right femur were examined histologically. The bone trabeculae in the vertebrae were markedly thickened, and the dimensions of the marrow spaces were correspondingly decreased. Along the edges of the trabeculae were narrow layers of newly deposited bone. The thick bone trabeculae had sinuous linear markings suggesting sequential cycles of bone deposition. The wide marrow spaces contained hematopoietic tissues; the small ones, edematous fibrous tissues with some plasma cells and lymphocytes or small foci of hematopoietic tissues. The changes in the ribs and in the right femur were similar.

Sections of the spleen and of the scarred lung tissues were stained with phosphotungstic acid-hematoxylin, Mallory's aniline blue, Van Gieson's stain and Weigert's elastic fiber stains and for iron. These preparations confirmed the statements made. The oxidase stains of the spleen demonstrated many granular cells in the myeloid tissues.

The generalized osteosclerosis noted in the bones of this patient with polycythemia vera probably is a change occurring in the advanced chronic stages of the disease. The stimulus of the myeloid tissue, whatever it may be, or the altered condition of the bone marrow accompanying the hyperplasia of the myeloid tissue, alone or together, caused this overgrowth of the bone substance. As the compartments of the marrow tissues were encroached on and decreased in size because of the thickening of the trabeculae, the demands for activity of extramedullary myeloid tissue increased.

2. DuBois, F. S.: Arch. Path. **12**:222, 1931.

Fig. 1.—Photomicrograph of the transverse section of a rib illustrating the marked overgrowth of bone and the small marrow spaces; × 8.

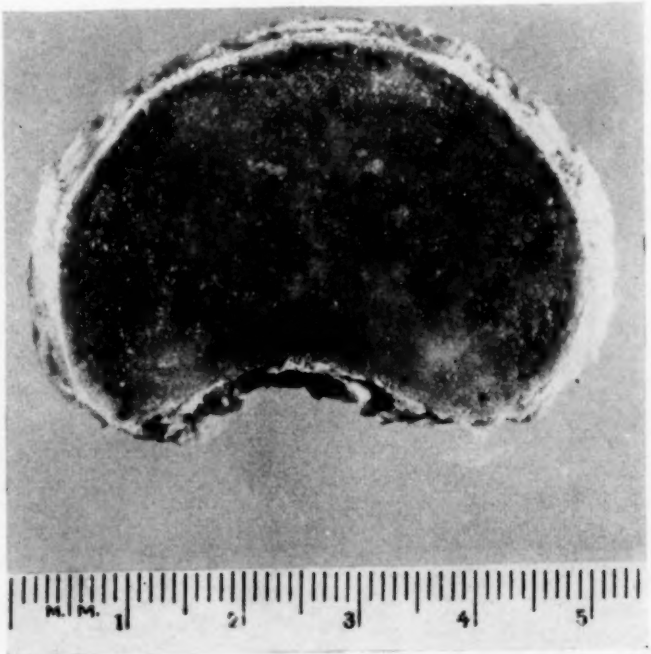


Fig. 2.—Photomicrograph of a sawed surface of the body of a lumbar vertebra. Note the marked osteosclerosis and the correspondingly decreased marrow.

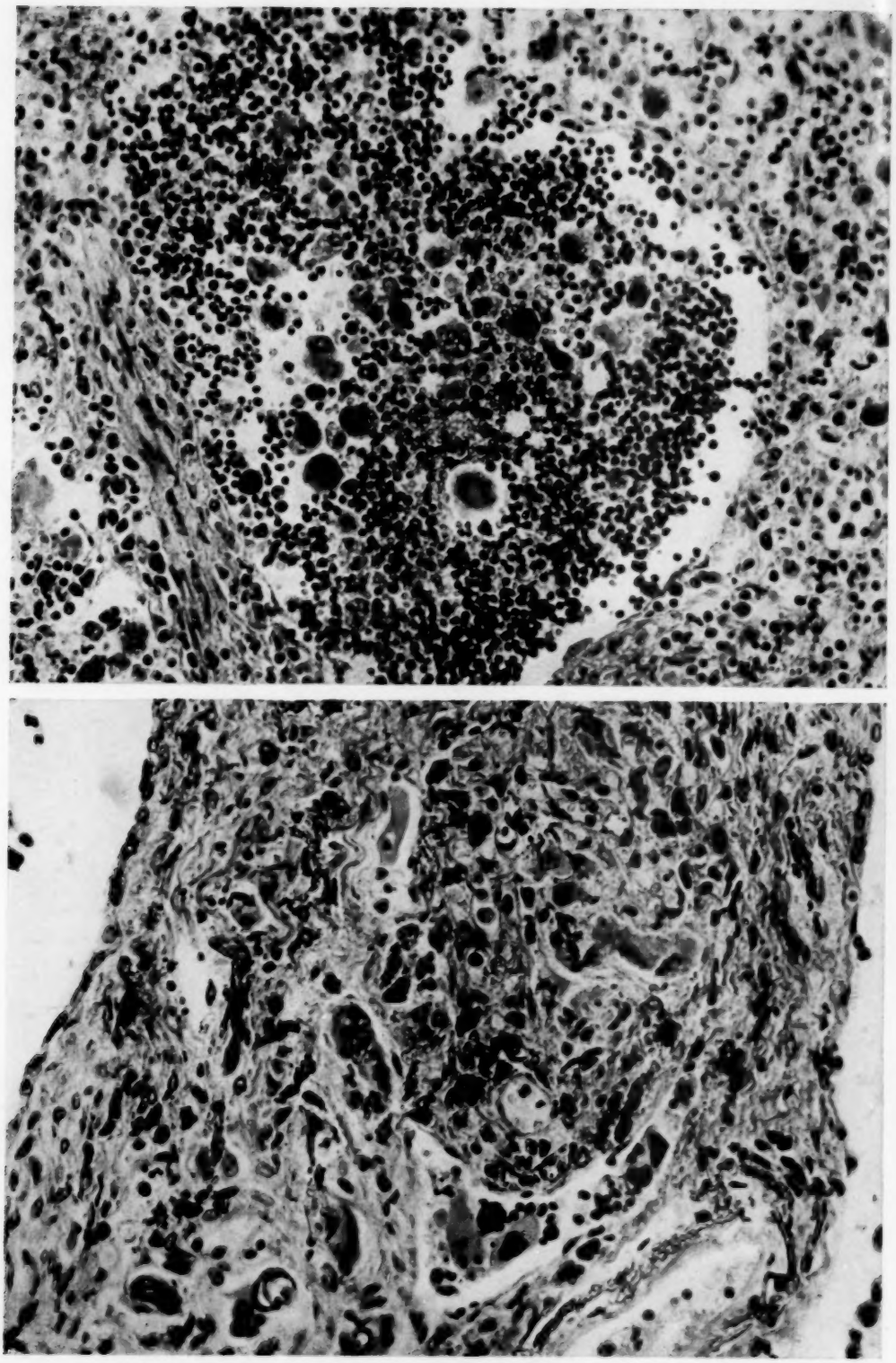


Fig. 3.—*A*, photomicrograph of a nodule of myeloid tissue in the spleen. The cells included elements of the erythroblastic and leukoblastic series and many megakaryocytes; × 270. *B*, photomicrograph illustrating scar tissues in the lung, the incrusted fibers, some foreign body giant cells and small masses of myeloid tissue; × 326.

SUMMARY

Marked sclerosis of the bones was observed in a patient whose polycythemia vera had been recognized clinically thirty-one years before death. These changes of the bones presumably occur in advanced stages of the disease. The linear markings of the thickened bone trabeculae indicate deposits of bone in growth cycles rather than continuously. The constricted marrow spaces in the bones were filled with hyperplastic myeloid tissues. Extramedullary foci of hyperplastic myeloid tissues occurred in the spleen, liver, lymph nodes and lungs.

Notes and News

Society News.—The Section on Pathology and Physiology of the American Medical Association has appointed the following section exhibit committee for the scientific exhibit at the Atlantic City session of the American Medical Association and the Canadian Medical Association: Frank W. Hartman, Detroit, chairman; A. B. Luckhardt, Chicago; E. P. Lyon, Minneapolis; J. P. Simonds, Chicago, and William Boyd, Winnipeg, Canada. Application for space must be made by Feb. 25, 1935. Application blanks may be obtained from any of the members of the Committee or from the Director, Scientific Exhibit, 535 North Dearborn Street, Chicago.

Harvard Committee on Research in Dental Medicine.—A representative committee of the faculties of Harvard University has been given general supervision of research in the dental school. A grant from the Milton Fund has been made for work on the effects of nutrition on teeth and their supporting structure. Among other functions this committee will assist the dental school in securing cooperation in research on problems that interest other departments also.

Registry of Tumors of the Central Nervous System.—The Harvey Cushing collection of about two thousand specimens of tumors of the central nervous system has been installed at Yale University, New Haven, Conn. The collection is fully catalogued. Any one who may wish to send specimens for diagnosis or seek other information, as, for example, that regarding the prognosis for any type of tumor, is invited to do so. An advisory board has been organized for the collection, the curator of which is Dr. Louise C. Eisenhardt.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

INTOXICATIONS AND MEGAMONONUCLEOSIS. S. NICOLAU, P. POINCLOUX, L. KOPCIOWSKA and G. BALMUS, Ann. Inst. Pasteur **52**:316, 1934.

A previous study of intoxication following burns is followed further by a specific study of what the authors call "megamononucleosis," characterized by an increase of large mononuclears, Türk and Rieder cells and young primordial cells. The normal figure in a rabbit was less than 4 per cent. It tripled with a single anesthetizing dose of chloralose. It doubled after an intramuscular injection of nupercaine. With bismuth, mercury, cobra venom and bacterial toxins the figure increased six times or more, after which it was reduced to normal in from eighteen to thirty hours. In the rabbit megamononucleosis is considered to be the chief leukocytic index of intoxication, just as leukocytosis accompanies acute bacterial infection, eosinophilia accompanies helminthiasis, and lymphocytosis goes with certain chronic infections. Besides the observations previously noted made on burned persons, the principle seemed confirmed in a patient recovering from tetanus, in one profoundly intoxicated with Trichocephalus and in several patients with diabetic gangrene.

M. S. MARSHALL.

EXPERIMENTAL ENDOMETRIOSIS. OTTO BRAKEMANN, Arch. f. Gynäk. **155**:276, 1933.

Under anesthesia the abdominal cavity of a rabbit was opened and pieces of the animal's uterine mucosa were sutured in various locations. This operation was performed in normal and castrated animals. In the presence of healthy ovaries the transplants grew well. The glands of the mucosa tended to dilate and form cysts. In the castrated animals growth of the transplant was poor; there were comparatively few glands. Extraperitoneal transplants between the inner borders of the rectus were also successful. Loose transplants tended to attach themselves to the pelvic viscera and to scar tissue. During heat the transplants increased in size. The pregnancy hormone seemed to exert no influence on the size of the autotransplant; however, the epithelial cells were changed in appearance.

JACOB KLEIN.

PATHOGENESIS OF ACUTE PANCREATIC NECROSIS. J. BALÓ, Beitr. z. path. Anat. u. z. allg. Path. **92**:14, 1933.

Acute diffuse necrosis of the pancreas is the result of activation of the pancreatic ferments. The condition is more common in obese persons than in others. In obese persons and fattened swine multiple minute fat necroses of the pancreas are not uncommon. Believing that their formation might shed light on the pathogenesis of diffuse pancreatic necrosis, Baló determined quantitatively the proteolytic, amylolytic and lipolytic activity of the pancreases of fattened swine and of two obese human beings. The enzymatic activity was found to be increased when compared with that of the pancreas of the unfattened swine. The activity of lipase was increased more than that of the other ferments. Lipase is excreted as an enzyme, not as a zymogen. Its greater activity in the pancreas of the obese person and of the fattened swine leads to the formation of the discrete small fat necroses not infrequently seen. These necroses may be the factor that activates the other pancreatic enzymes and leads to diffuse necrosis.

O. T. SCHULTZ.

EFFECT OF THYROXINE ON THE THYMUS. HANNA SCHULZE, Beitr. z. path. Anat. u. z. allg. Path. **92**:329, 1934.

To determine the interrelations of the thyroid and thymus, new-born white mice were given daily subcutaneous injections of thyroxine beginning on the day after birth. The effect on the thymus was determined at varying periods after birth. There was an absolute as well as a relative decrease in the size of the thymus. When the injections of thyroxine were not begun until the thirteenth day of life thymic atrophy was less marked. In comparable experiments on new-born white mice, rabbits and kittens the two last named species were found to be less resistant to the toxic action of thyroxine, and a lesser degree of atrophy of the thymus resulted. The simultaneous injection of thyroxine and an extract of the thymus enhanced the toxic action of thyroxine.

O. T. SCHULTZ.

EXPERIMENTAL ACUTE MORPHINE GASTRITIS. H. HANKE, Beitr. z. path. Anat. u. z. allg. Path. **92**:390, 1933.

The subcutaneous injection of morphine into cats was followed by the excitation characteristic of the action of this drug in cats and by death within a few hours. The motility of the stomach was decreased and the acidity of the gastric juice increased. The mucosa, especially of the body of the stomach, revealed numerous minute erosions, sometimes visible to the naked eye. In the lesions the surface epithelium was absent, and the floor of the defect was covered by a thin layer of tissue in a state of fibrinoid degeneration. Leukocytic infiltration was present in some of the lesions. The erosions are ascribed to the surface action of the acid gastric juice. The findings are interpreted as a confirmation of the peptic origin of acute gastric erosions.

O. T. SCHULTZ.

OXIDASE REACTION IN THE MONOCYTES OF RABBITS. W. NAGEL and W. BÜNGELER, Frankfurt. Ztschr. f. Path. **45**:403, 1933.

While the monocytes of rabbits usually give a negative oxidase reaction, a positive reaction can be brought about by the injection of a material containing oxidase-positive granules (radish juice) into the portal circulation. Not only the monocytes in the hepatic vessels will reveal oxidase-positive granules, but also the capillary endothelial cells of the liver. Thus the intimate relationship between the reticulo-endothelium and the monocytes is supported, and further evidence is added to the authors' previous view that the monocytes are derived from reticulo-endothelium. They conclude that the occasional occurrence of a positive oxidase reaction in monocytes should not be traced to a possible myelogenous nature of these cells but, more likely, to a phagocytosis of oxidase-positive material by these cells.

W. SAPHIR.

ARE CAPILLARIES FORMED IN TISSUE CULTURES OF LEUKOCYTES? H. SALFELD, Frankfurt. Ztschr. f. Path. **45**:414, 1933.

In five hundred tissue cultures made of chicken blood plasma or plasma and embryonic extracts, no evidence was found of a formation of true capillaries. Attention is called to structures somewhat resembling capillaries.

W. SAPHIR.

EXPERIMENTAL OSTEITIS FIBROSA IN RATS. G. GAEHTGENS, Frankfurt. Ztschr. f. Path. **45**:543, 1933.

The present report is based on the researches of Rutishauser and Katase, who produced osteitis fibrosa in rabbits by causing an acidosis with lead acetate, dextrose solutions and anesthesia. The work was undertaken to ascertain whether or not the disease could be produced in smaller animals. With lead acetate, Gaehtgens reports the production of osteitis fibrosa in rats but not in mice. With ether

anesthesia there was no change in the skeletal system of mice, and only a beginning osteitis in rats. Dextrose administered subcutaneously produced no changes in either animal. He feels in accordance with other workers that acidosis disturbs calcium exchange and causes depletion of the calcium content of bones. Osteitis fibrosa is the result of the reaction of the surrounding structures toward the primary calcium deprivation.

H. Horn.

Erythropoiesis in Tissue Culture. A. von Jeney, Virchows Arch. f. path. Anat. **290**:675, 1933.

In previous work it had been possible to observe the development of erythrocytes to the normoblast stage under the influence of substances added to the tissue culture. In the present experiments various chemicals were added to cultures of rabbit bone marrow with the aim of inducing the formation of ripe red corpuscles. Globin led to increased formation of primitive cells, its action being similar to that of liver extract. Hematin and the iron-free precursors of hemoglobin in the presence of iron caused the formation of normoblasts. Proline had the same effect. Increased numbers of non-nucleated erythrocytes were seen when histidine and arginine were used; the maturation of the erythrocytes is ascribed to the action of the hexone bases, although it is not possible to exclude entirely the preservation of original red corpuscles by these substances. Tryptophan and cystine increased the maturing action of histidine. Pyrrole was apparently stored by the tissue and aided the formation of hemoglobin. Asparaginic acid increased granulopoiesis.

O. T. Schultz.

The Influence of Butter Fat and Peanut Butter Oil on the Growth and Fertility of Rats. H. Krieger Lassen, Acta path. et microbiol. Scandinav. **11**:183, 1934.

Histologic Investigation of Rats on Butter Diet. A. Ringsted, Acta path. et microbiol. Scandinav. **11**:197, 1934.

Peanut oil with the addition of cod liver oil gives just as good a growth of rats as butter fat in a diet consisting of 20 per cent casein, 5 per cent salts, 60 per cent rice starch and 15 per cent fat. Peanut oil permits good fertility, whereas in every case butter fat counteracts fertility in female rats, reducing it in males.

Microscopically, "oil males" and "oil females" showed normal internal genitals with the exception of one single "oil male" which showed moderate degenerative changes of the testis. In three cases the "butter females" presented signs of being pregnant or of having been so. In the "butter males" marked degenerative changes of testes were found in all the animals which had not had an addition of a wheat germ oil preparation to their diet and, besides, in two of those which had had this preparation, whereas three of the "butter males" of the latter group presented varying degrees of protection of the germinal epithelium. The agreement of the course of gestation in the "butter females" and the marked degenerative changes of the testes of the "butter males," respectively, and the phenomena characteristic of E avitaminosis is pointed out.

Pathologic Anatomy

Ossification and Calcification of the Dura Mater. R. Bonnard, Ann. d'anat. path. **10**:55, 1933.

Bonnard analyzes the historical, clinical, roentgenologic, and pathologic aspects of calcification of the dura mater, including a number of cases recorded in the literature as well as a personal case. The condition is rare, diffuse or localized, and symptomless, being usually an accidental finding at autopsy or on roentgenography. The localized forms occurring in the falx cerebri are roentgenologically distinct. Their etiology is unknown. The calcifications are interstitial, between

the two layers of the dura, and have nothing to do with calcified plaques on the outside of the dura next to the skull, with calcification occurring in external or internal pachymeningitis or with other similar conditions. Such calcification is normal among certain lower animals, and in man may represent an atavistic resumption of this property.

PERRY J. MELNICK.

ANATOMIC FORMS OF PULMONARY TUBERCULOSIS. F. BEZANÇON, G. ROUSSY, C. OBERLING and J. DELARUE, Ann. d'anat. path. **10**:105, 1933.

Recent biologic, bacteriologic and clinical studies of pulmonary tuberculosis have to a certain extent forced the study of its pathologic anatomy into the background. The authors feel that studies of the morbid anatomy can still offer contributions of value. They have constructed a classification of the anatomic forms of pulmonary tuberculosis on the basis of (*a*) the histologic structure of the lesions (exudative, proliferative and fibrotic) and (*b*) their distribution and topography (miliary, nodose and diffuse).

Five types of lesions (simple forms) are described, namely: lobar, nodose, acinous, infundibular and interstitial. These rarely occur as isolated forms; usually several types of lesions are found simultaneously (complex forms). The lobar types are usually exudative (noncaseous or caseous), rarely proliferative and still more rarely fibrotic (i. e., after collapse therapy). The nodose types (confluent acinous) are either disseminated (i. e., in galloping consumption) or localized (in more chronic cases). Acinous types are similarly either disseminated (usually in children) or localized (as in chronic apical lesions). Infundibular types are usually disseminated, namely, miliary tuberculosis. They may be exudative, proliferative or fibrotic. Interstitial forms are usually chronic, peribronchial or interacinous, and lead to sclerosis or to atelectasis or emphysema. The difficulty of constructing a purely anatomic classification is emphasized.

PERRY J. MELNICK.

THE HISTOLOGIC LESIONS OF RHEUMATISM. E. C. CRACIUN, N. VISINEANU, N. GINGOLD and A. URSU, Ann. d'anat. path. **10**:157, 1933.

The article is a general review presenting first the various theories of the etiology of rheumatism, namely, theories relating to infections and allergic conditions, followed by a consideration of the histologic changes which have been described in different organs. The histologic observations reported by various authors in the heart, vessels, muscles, connective tissues, serous membranes, joints, intestine, seminal vesicles, liver, lung, brain and amyloid infiltrations are discussed. The only specific findings are the Aschoff nodules in the heart. In other organs the changes, although not specific, are characteristic of rheumatism. There is a good bibliography. The authors mention characteristic findings in the kidney which they themselves have discovered, which they report in a separate paper.

PERRY J. MELNICK.

DUODENAL STENOSIS RESULTING FROM MALFORMATION OF THE HILUS OF THE LIVER. H. L. ROCHER, G. ROUDIL and J. COURRIADES, Ann. d'anat. path. **10**:277, 1933.

In an infant 10 days old, with pyloric stenosis, who died following gastroenterostomy autopsy revealed as causing the stenosis a number of peculiar congenital malformations of the hilus of the liver. In particular, the portal vein had an anomalous course anterior to the duodenum, compressing it. The three vitellovitelline anastomoses in the embryo are discussed, and the condition in the present case is traced to a failure of atrophy of the various elements in the normal manner.

PERRY J. MELNICK.

RENAL LOCALIZATION OF RHEUMATISM. E. C. CRACIUN, N. VISINEANU, N. GINGOLD and A. URSU, Ann. d'anat. path. **10**:363, 1933.

In seven of fifteen persons who died during an attack of rheumatic fever the authors found characteristic renal changes. These consisted of an acute proliferative and desquamative extracapillary glomerulonephritis which led to obliteration of Bowman's space and gradual scarring of the glomeruli. The lesion was not extensive, picking out only a few glomeruli. The clinical findings were therefore minimal, but according to the authors the gradual obliteration of the glomeruli after a long time or after repeated attacks might result in Bright's disease. The findings were acute in one case; in the others, subacute or chronic. The first case had the most clearcut changes; in the others they were differentiated with difficulty from ordinary glomerulonephritis. The lesions are not specific but are considered to be characteristic by the authors.

PERRY J. MELNICK.

ENDOMETRIOSIS. L. CORNIL, M. MOSINGER and X. FRUCTUS, Ann. d'anat. path. **10**:389, 1933.

The authors present a discussion of tubal endometriosis based on a study of eight new cases. Three possible etiologies are presented. An inflammatory basis accounts for several cases, either by producing diverticulum-like processes in the cornua (nodose isthmic salpingitis) or by a direct metaplasia such as is often seen in the inflammatory states. Misplaced embryonic rests undoubtedly account for some cases. The process results from heterotopia of the müllerian duct, not of the wolffian duct. Hyperplastic states, probably under ovarian influence, form a third type. Migration of endometrial fragments is rejected as a possible etiology.

PERRY J. MELNICK.

NASAL POLYPS. L. LEROUX and J. DELARUE, Ann. d'anat. path. **10**:879, 1933.

The authors classify nasal polyps into four groups. Each group is characterized by certain clinical and histologic features. 1. Inflammatory nasal polyps are composed of inflammatory granulation tissue, often very vascular, with extensive round cell and plasma cell infiltrations. This form has as its background a chronic infection, usually of the accessory nasal sinuses. 2. Edematous nasal polyps, the stroma of which is edematous, present a pseudomyxomatous picture. They are found in patients with vasomotor and sympathetic instability, vasomotor rhinitis, etc. 3. A third group of polyps has an extensive infiltration of the stroma by eosinophil leukocytes. This form occurs in patients with asthma and other allergic phenomena. 4. The tumor polyps may be composed of either a glandular or an epithelial proliferation; rarely an angiomatous picture is seen, but in the latter case the polyp is usually on an inflammatory basis.

PERRY J. MELNICK.

TERTIARY SYPHILIS OF THE LUNG. K. SCRIBA AND H. E. BÜTTNER, Virchows Arch. f. path. Anat. **291**:571, 1933.

A man aged 56 had had symptoms of chronic pulmonary disease for two years previous to his death. The Wassermann reaction was strongly positive. Roentgenograms taken at intervals during a period of eighteen months revealed nodular fibrosis of the lower lobes and multiple small miliary nodules of the upper lobes of the lungs. Anatomically the process was characterized by nodular and somewhat diffuse sclerosis of the lungs, which was most marked in the lower lobes, and by miliary gummas in various stages from an early phase to fibrosis. The continuous and progressive development of miliary gummas, evident both roentgenographically and anatomically, is considered the unusual feature of the case. The combination of miliary gumma formation and nodular sclerosis is held to be the result of the interaction of syphilis and pneumonoconiosis.

O. T. SCHULTZ.

THE RETICULO-ENDOTHELIAL TISSUE OF THE BRAIN IN SENILE PSYCHOSES. W. K. BELEZKY AND E. I. JERMOLENKO, Virchows Arch. f. path. Anat. **291**:607, 1933.

Belezky is a proponent of the view that a not inconsiderable portion of the glia is mesenchymal in origin. He and Jermolenko make several assumptions: that elderly people are more susceptible to infection than younger persons, that this greater susceptibility is due to atrophy of the reticulo-endothelial system, and that elderly persons with senile psychoses are more prone to infection than elderly people without such psychoses. They then set up the thesis that the mesenchymal glia or mesoglia, which they consider part of the reticulo-endothelial system, should present morphologic evidence of alteration. The brains of eleven persons who suffered from senile psychosis, all but one having an associated cerebral arteriosclerosis and the brains of a person with presenile psychosis and two persons with manic depressive psychosis were examined histologically. They conclude that the mesoglia in persons with senile psychosis is atrophic and reacts very slightly to infection, whereas previous studies have shown that the mesoglia of young persons takes an active part in the reaction to infection. In senile psychosis the glia of ectodermal origin, the ectoglia, is hypertrophied and hyperplastic. They claim to have demonstrated for the first time fibrosis of mesenchymal origin in the cerebral parenchyma. The plaques of senile psychosis are the result of mesoglial fibrosis. Ectoglial fibrosis also occurs in senile psychosis.

O. T. SCHULTZ.

ADAPTIVE CHANGES IN THE CARTILAGE AND BONE OF ANKYLOSED JOINTS. S. M. RABSON, Virchows Arch. f. path. Anat. **291**:624, 1933.

Normal bone and cartilage are closely adapted in their structure and in the amount of their material to the functional, that is mechanical, demands made of them. Rabson made a microscopic study of the cartilage and bone of two knee joints that had been ankylosed as the result of previous inflammation. In such joints there are areas of nonfunction and other areas of abnormal function. Rabson found that complete cessation of function led to atrophy and disappearance of cartilage and to thinning of the bony trabeculae of the spongy bone. When function is reestablished through a partial return of motion, the cartilage regenerates and the osseous trabeculae of the spongy bone of the epiphysis increase in thickness. Abnormal and excessive function also lead to disappearance of cartilage.

O. T. SCHULTZ.

CHANGES IN THE GASSERIAN GANGLION IN RHEUMATIC INFECTION. E. JUNGHANS, Virchows Arch. f. path. Anat. **291**:643, 1933.

This is the fifteenth in the series of contributions on rheumatic fever emanating from the pathologic institute of the University of Leipzig. Both trigeminal ganglions were removed at necropsy and examined histologically, special attention being paid to inflammatory changes in the stroma and vessels. The material studied came from nine persons with certain late rheumatic infection, ten with endocarditis but no anatomic findings to indicate that the process was of rheumatic origin, three with trigeminal neuralgia and a series of controls without evidence of rheumatism. In all of the cases of certain rheumatic infection subacute and chronic inflammatory reaction of variable degree was noted in the ganglions. In the cases of nonrheumatic endocarditis similar changes were very slight or absent. In the three clinical cases of trifacial neuralgia the ganglions revealed well marked to severe changes like those noted in rheumatism; furthermore, anatomic evidence of previous rheumatic infection, unsuspected clinically, was uncovered at necropsy. In the control series inflammatory reaction of low grade was seen only in three cases of sepsis. Junghans concludes that rheumatic infection causes changes in the vessels and stroma of the gasserian ganglion identical with those caused by this infection elsewhere in the body. These alterations need not lead to clinical symptoms of neuralgia. However, the presence of similar alterations in three

clinical cases of trifacial neuralgia, together with evidence of previous rheumatic infection, is strong presumptive proof that rheumatic infection may cause trifacial neuralgia.

O. T. SCHULTZ.

LATENT FIBROUS OSTEODYSTROPHY IN HYPERTHYROIDISM. M. ASKANAZY AND E. RUTISHAUSER, Virchows Arch. f. path. Anat. **291**:653, 1933.

The association of hyperthyroidism and osteomalacia, as reported in the literature, led to the histologic study of the bones in seven fatal cases of exophthalmic goiter. The authors confirm the occurrence of skeletal changes in this disease. The changes noted were not those characteristic of osteomalacia. The process is one of bone atrophy, with osteoclastic absorption of bone and its secondary replacement by connective tissue. The alterations noted are those of mild or latent fibrous osteodystrophy. The changes were best seen in those portions of the skeleton subjected to active functional demands. Similar alterations were observed in animals (rabbits and cats) that received thyroxine or desiccated thyroid substance. The process is not the result of parathyroid abnormality. In both the human subjects and the experimental animals the parathyroid glands were normal or slightly hyperplastic, the latter state being held secondary to the disturbed calcium metabolism. The osseous changes of hyperthyroidism are the result of abnormal thyroid function.

O. T. SCHULTZ.

TONOGENIC DILATATION OF THE RIGHT SIDE OF THE HEART. E. KIRCH, Virchows Arch. f. path. Anat. **291**:682, 1933.

The dilatation of the right ventricle that results from increased mechanical resistance to the flow of blood through the pulmonary circuit Kirch terms "tonogenic," in contrast to the dilatation due to weakness or disease of the myocardium, which he terms "myogenic." Tonogenic dilatation occurs chiefly in the long axis of the ventricle, whereas myogenic dilatation occurs in the transverse diameter. The characteristics of tonogenic dilatation of the right ventricle of the human heart Kirch has previously described. In this paper he summarizes experimental work on dogs, cats, rabbits and guinea-pigs, undertaken to elucidate the development and course of this form of dilatation. Obstruction to the pulmonary circulation was produced by injection of metallic mercury into the pulmonary circuit. Dilatation was observed first in the pulmonary conus, which is the terminal portion of the path of outflow from the ventricle. The conus normally holds the residual blood at the end of systole; hindrance to the outflow increases the residuum and dilates the conus in its longitudinal axis, the course of the muscle fibers being such that mechanical dilatation occurs more readily in the long axis. With continued hindrance to outflow the dilatation proceeds in retrograde fashion along the path of outflow to the apex, finally involving the path of inflow and the auricle. In tonogenic dilatation of the right ventricle the heart is rotated to the left about its longitudinal axis. Recovery from tonogenic dilatation is complete if the obstruction to the pulmonary circulation disappears.

O. T. SCHULTZ.

LYMPHOCYTIC INFILTRATION OF THE PERIPORTAL TISSUE OF THE LIVER. L. KETTLER, Virchows Arch. f. path. Anat. **291**:706, 1933.

Slight periportal lymphocytic infiltration of the liver was observed in nine persons free from any evidence of disease other than that resulting from the accident or other form of violence that led to sudden death. The cellular accumulations bore no relation to the age of the victim. Lymphoid follicles were never observed. The cellular infiltration was most marked in cases of infection and sepsis, especially if acute splenic tumor was present. Such infiltrations bore no detectable relation to alterations in the hepatic cells. They are held to be the result of an inflammatory reaction to bacterial toxins.

O. T. SCHULTZ.

FETAL PERITONITIS DUE TO APPENDICITIS. R. KNEPPER, Virchows Arch. f. path. Anat. **291**:741, 1933.

A child who died at the age of 2½ months began to lose weight rapidly at the eighth week of life, developed diarrhea and fever, became dehydrated and died. Necropsy revealed the presence of organized fibrous peritoneal adhesions, with an encapsulated mass of exudate in the ileocecal region. The distal end of the appendix was open and was embedded in the mass of exudate. The latter was partly calcified and contained meconium.

O. T. SCHULTZ.

CONGENITAL INSUFFICIENCY OF THE TRICUSPID VALVE. H. GÖTZ, Virchows Arch. f. path. Anat. **291**:835, 1933.

The anomaly described was observed in the heart of a child who died at the age of 8 months. The insufficiency of the valve was due to downward misplacement of the medial and posterior segments, the shape of which was also abnormal. The anterior segment was normal in shape and position, but lacked cordae tendineae and papillary muscles and was broadly united to the wall of the ventricle. The medial segment was similarly devoid of cordae tendineae and papillary muscles. These structures were present for the posterior segment, but the latter was perforated at its middle. Endocardium was absent or incompletely developed in portions of the ventricle. It is to the defective development of the endocardium that the valvular anomaly is ascribed.

O. T. SCHULTZ.

HISTOPATHOLOGY OF THE CUTANEOUS NODULES CAUSED BY FILARIA VOLVULUS. W. FISCHER, Virchows Arch. f. path. Anat. **291**:854, 1933.

Fischer describes and illustrates the histology of the lesions of the skin caused by Onchocerca (Filaria) volvulus. His material came from Africa, where the infection is widespread and prevalent. The larva or microfilaria is transmitted by the biting insect Simulium damnosum. A histologically identical infection is very prevalent in Guatemala and Mexico and is caused by the species Onchocerca coecutiens.

O. T. SCHULTZ.

HISTOLOGY OF UNDULANT FEVER. L. HASLHOFER, Virchows Arch. f. path. Anat. **291**:912, 1933.

In a child, aged 3½ years, who died of undulant fever of three months' duration, the essential alteration was cellular and fibrillar hyperplasia of the reticulum of the follicles of the lymph nodes and spleen. The reaction was a granulomatous one, with the presence of plasma cells and multinucleated giant cells.

O. T. SCHULTZ.

CHANGES IN THE INTESTINE FOLLOWING EXTENSIVE RESECTION OF THE SMALL INTESTINE. F. BORNSTEIN, Virchows Arch. f. path. Anat. **291**:921, 1933.

A woman aged 29 years at the time of her death had undergone resection of 530 cm. of the small intestine eleven years previously. Most of the ileum and a large part of the jejunum had been removed. At death the most striking feature of the remnant of small intestine and of the large intestine was a great increase in the size and number of the Kerkring's folds of the mucosa. The cellular elements of the mucosa were hypertrophied. These changes are held to be compensatory, their purpose being to increase the absorptive surface of the intestine.

O. T. SCHULTZ.

FLUORESCENT MICROSCOPY OF HUMAN TISSUES. H. HAMPERL, Virchows Arch. f. path. Anat. **292**:1, 1934.

When tissue sections are examined by ultraviolet rays they exhibit varying degrees of fluorescence. This is a property, not of the homogeneous nucleoplasm

or cytoplasm, but of materials in the plasm. Hamperl describes the technic of ultraviolet microscopy and presents the results of a systematic study of various tissues and organs by this method.

O. T. SCHULTZ.

INVAGINATION OF THE COMMON DUCT INTO THE DUODENUM. H. WISCH, Virchows Arch. f. path. Anat. **292**:71, 1934.

As a condition not previously described, Wisch reports an invagination of the common duct into the duodenum. The condition occurred in a woman aged 52 years. The onset was sudden, with cardiac disturbance and rapidly developing jaundice. There was no biliary colic. Death occurred after ten months of progressive loss of weight and strength and continuous jaundice. The feces were not clay-colored. At necropsy the terminal portion of the common duct was found invaginated into the duodenum; the papilla was elongated to 1 cm. The submucous tissue of the papilla and duct was looser than normal, but no cause for this state could be found. There were no gallstones.

O. T. SCHULTZ.

HISTOLOGIC CHANGES IN THE RADICLES OF THE PORTAL VEIN IN CIRRHOSIS OF THE LIVER. J. WEEBER, Virchows Arch. f. path. Anat. **292**:75, 1934.

The radicles of the portal vein in the omentum, mesentery, intestine and stomach were examined microscopically in twenty-three cases of Laënnec's cirrhosis and three cases of Hanot's cirrhosis. Similar studies were made in ten cases of heart failure with passive congestion and ascites and in ten control cases. In cirrhosis the media of the portal radicles was hypertrophied, sometimes to two and a half times the normal thickness. A similar condition was observed in heart failure with portal stasis. The medial hypertrophy is held to be compensatory in origin. In interlobular cirrhosis without portal congestion or distention of esophageal veins, the portal radicles revealed only slight change. In cirrhosis with hypertrophy of the media of the portal radicles, these vessels revealed also nodular or ridgelike thickenings of the intima, which were free from degenerative changes. The intimal thickenings are mechanical in origin.

O. T. SCHULTZ.

LIPOID DEPOSITION IN TENDONS. FANNY CHALETZKAJA, Virchows Arch. f. path. Anat. **292**:85, 1934.

The basis of this work is the thesis of the Anitschkow school that atherosclerosis is a systemic disease of the fibro-elastic tissues of the body. In the deposition of lipoids in the aorta the important factors are the character of the chondromucoid chromotropic ground substance and the lymph supply of the tissues. The structure, lymphatic nutrition and ground substance of tendons are held to be closely similar to those of the wall of the aorta. The tendons of forty-eight persons, subjects of necropsy, were examined histologically by means of the sudan III stain. The ages varied from 3 to 82 years. The tendons of the upper and lower extremities were examined. Lipoid deposition was not observed before the fourth decade. Beginning at this age period, it became progressively more marked with advancing years, and was always most extreme in the achilles tendon. It occurred first as fine granules and later as larger and coarser masses. It was at first isotropic and later anisotropic and then contained cholesterol crystals. Lipoidosis of tendons was most marked in persons with advanced atherosclerosis, but in some of those with atherosclerosis it was not any more extreme than in other persons of the same age. In elderly persons there was observed also the deposition of homogeneous colloid protein masses in the tendons. Lipoid deposition was not usually associated with reactive changes. The deposition was the same in comparable tendons of the two sides of the body and bore no relation to occupation. Lipoidosis of tendons is looked on as a local manifestation of a generalized infiltrative process that depends on disturbed cholesterol metabolism. The importance of the findings in the study of the nature of atherosclerosis and of abnormal lipoid metabolism in general is stressed.

O. T. SCHULTZ.

EFFECTS OF HIGH ENVIRONMENTAL TEMPERATURE ON THE PERIPHERAL BLOOD PICTURE. N. SASYBIN, Virchows Arch. f. path. Anat. **292**:86, 1934.

Studies of the peripheral blood were made in twenty-five workers who had been exposed for variable periods to high temperatures and of seventeen dogs and three cats which had been exposed to temperatures of from 35 to 60 C. for periods of from two and one-half hours to thirty-five days. Short exposures caused an increase in number of both erythrocytes and leukocytes of between 20 and 40 per cent. The duration of the action of heat appeared to be a more potent factor than the degree of heat. After several days' exposure the erythrocytes dropped to between 20 and 50 per cent below the initial figure and the leukocytes increased to between two and five times the original number. With still more prolonged action there was a steady and progressive decrease in the erythrocytes and a continuous increase in the leukocytes. Thus, in an experiment of sixteen days' duration the erythrocyte count decreased from 5,600,000 to 900,000, and the hemoglobin from 90 per cent to 20 per cent; the leukocyte count increased from 6,700 to 50,000. Accompanying the quantitative alterations in the cellular elements were morphologic changes as follows: faint staining of erythrocytes and the appearance of erythroblasts; increase in the various kinds of leukocytes, especially in the monocytes; a shift to the left of the neutrophil granulocytes; the appearance of myelocytes; karyorrhexis of lymphocytes and granulocytes; karyokinesis of monocytes and erythrophagocytosis by these cells; vacuolation and degeneration of the cytoplasm of the leukocytes.

O. T. SCHULTZ.

EFFECTS OF HIGH ENVIRONMENTAL TEMPERATURE ON THE HEMATOPOIETIC SYSTEM. N. SASYBIN, Virchows Arch. f. path. Anat. **292**:114, 1934.

Histologic study was made of the hematopoietic tissues of seventeen dogs and three cats which had been subjected to temperatures of from 35 to 60 C. for periods of from two and one-half hours to thirty-five days. After short exposure, during which the animal was able to adjust itself to the harmful action of heat, the reticulo-endothelial elements of the lymph nodes, spleen, bone marrow and liver revealed increased activity, as evidenced by increase in the size of the cells and by erythrophagocytosis. In the second stage of incomplete or failing compensation hyperplasia of the reticulo-endothelial system was marked. The isolation of monocytes from the syncytium of the lymph nodes was observed. The spleen was enlarged and fleshy. The bone marrow was red. After exposure so prolonged that it lead to the death of the animal, the bone marrow consisted largely of erythroblasts and myeloblasts. Extramedullary hematopoiesis was widespread. Myelopoiesis predominated over erythropoiesis. Fragmentation of lymphocytes was observed.

O. T. SCHULTZ.

RELATIONSHIP OF THE POLYCYSTIC PANCREAS TO THE POLYCYSTIC KIDNEY AND LIVER AND TO ANOMALIES OF THE NERVOUS SYSTEM. E. RÜMLER, Virchows Arch. f. path. Anat. **292**:151, 1934.

Rümler describes seven cases that form the basis of a discussion on the relationships of the polycystic pancreas, kidney and liver. In a premature stillborn and a new-born infant exencephaly was associated with a polycystic condition of the kidney, liver and pancreas and a cyst of the eyelid in one instance and with a similar condition of the kidey and liver and a cyst of the retina in the other. In another new-born infant cysts in the kidney and liver were associated. The remaining cases, all in adults, included one of cysts in the kidney and liver, another of cysts of the kidney and pancreas and angioma of the retina and cerebellum, another of cysts of the pancreas and angioma of the cerebellum, and one of cysts in the kidney and pancreas and angioma of the cerebellum. The cystic state of the internal organs is ascribed to embryonic excess maldevelopment involving both the mesodermal connective tissue and the epithelium. In five cases epithelial overgrowth had led to the formation of intracystic adenomas. The relatively

frequent association of exencephaly, cyst of the retina and angioma of the cerebellum or retina indicates that the developmental anomaly is not necessarily limited to the kidney, liver and pancreas.

O. T. SCHULTZ.

MYOCARDIAL CHANGES FOLLOWING EXPERIMENTAL LIGATION OF THE CORONARY ARTERY. I. M. ICHTEIMANN, Virchows Arch. f. path. Anat. **292**:187, 1934.

The anterior descending branch of the left coronary artery was ligated in thirteen rabbits. The animals were killed at intervals of two hours to forty-five days. In the experiments of shorter duration the animal was vitally stained with trypan blue previous to ligation of the artery; in experiments of longer duration vital staining was undertaken during a period of a week preceding the killing of the animal. The immediate effect of the ligation was necrosis of the myocardium. The areas of necrosis were multiple, of various sizes and of different ages. They occurred not only in the wall of the left ventricle and in the septum, but also in the adjacent portions of the wall of the right ventricle. Removal of the necrotic material was effected chiefly by vitally staining histiocytes and to only a slight degree by polymorphonuclear leukocytes. The histiocytes were derived chiefly from the resting cells of the epicardium and perivascular tissue, and to a lesser degree from inwandering monocytes. Organization of the injured myocardium occurred by means of granulation tissue originating from the epicardium, the perivascular tissue and the intermuscular stroma. In this process the histiocytes did not take an active part; they became elongated and assumed their resting state. The cells that some have described as myocytes and that have been supposed to take part in regeneration of the myocardium the author holds to be histiocytes. He saw no evidence of regeneration of muscle, although hypertrophy of surviving muscle fibers was noted.

O. T. SCHULTZ.

A PECULIAR CASE OF INTERSEX IN MAN. E. W. THURSTON, Virchows Arch. f. path. Anat. **292**:220, 1934.

Since the time of birth doubt had existed as to the sex of the child. At the age of 13 years, a laparotomy was made to determine the nature of a tumor of the lower part of the abdomen. The patient presented the appearance of a young man with a light growth of hair on the upper lip and somewhat coarser hair on the chin. The mammae were not enlarged. The pubic hair showed a feminine distribution. There was a penis without a urethra at the usual location. Posteriorly were two labia-like folds of skin between which lay two openings separated by a short bridge of skin. A soft resistance was felt in the end of the right inguinal canal. Internally were found a vagina, which was continuous with the posterior of the two openings, a uterus and two tubes. The left tube ended in a mass of calcified material in the left flank. At the end of the right tube was found a testis-like organ lying in the right inguinal canal. In addition there was found a vas deferens on the right, bound with connective tissue to the tube and passing only as far as the posterior surface of the uterus, after which it disappeared. The vas deferens was not located on the left side. The epididymis on the right was well developed, but that on the left was atrophic. The testis-like gonad revealed a somewhat thick tunica albuginea and tubules with several layers of indifferent cells lying on a hyaline membrane. The interstitial tissue was rich in cells closely resembling Leydig cells. The stroma subjacent to the tunica showed a different character and closely resembled ovarian stroma. Serial sections failed to show either follicles or remains of them. Thurston believes that the explanation of so-called hermaphrodism lies in the genetic theory of R. Goldschmidt. A review of the theory brings out especially the point that hermaphrodites are persons whose genetic constitution should make them differentiate in the direction of one sex but who swing to a disturbance in the equilibrium of masculine and feminine determiners. This differentiation proceeds only to a certain point in the one direction, after which time the differentiation begins anew in the direction of the opposite

sex. An attempt is made to analyze the present case according to the Goldschmidt theory. By bringing into consideration the embryonic development of the male and female reproductive apparatus, the approximate time at which the change in the direction of differentiation occurred is established. He concludes after this analysis that the person in question began life genetically constituted to complete differentiation as a male, but ended differentiation as an intersex, showing both male and female sexual characteristics.

AUTHOR'S ABSTRACT.

FORMATIVE RELATIONS OF EPITHELIUM AND MESENCHYME. L. DOLJANSKI and F. ROULET, Virchows Arch. f. path. Anat. **292**:256, 1934.

In tissue cultures of liver the hepatic cells grow in the form of sheets of cells all of which are of similar morphology. In cultures containing mesenchyme the latter grows into the proliferating liver tissue in the form of connective tissue strands that subdivide the tissue. When a small group of hepatic cells becomes surrounded by connective tissue, they orient themselves about a lumen and become cuboidal or columnar. In this way bile ducts are formed in the tissue culture. The mesenchyme exerts a formative stimulus on the liver tissue and causes the latter to differentiate into bile duct epithelium. In pathologic states of the liver that result in the formation of so-called new-formed ducts or pseudoducts, the process is held to be essentially identical. In this process, too, it is the connective tissue that invades the hepatic lobule and exerts a formative influence on the liver cords and transforms them into bile ducts.

O. T. SCHULTZ.

HISTOLOGIC CHANGES IN THE ANTERIOR LOBE OF THE HUMAN HYPOPHYSIS FOLLOWING ITS IRRADIATION WITH ROENTGEN RAYS. E. STÖCKL, Zentralbl. f. Gynäk. **58**:1160, 1934.

Thirty-eight days after roentgen irradiation of the hypophysis extensive necrosis was found involving about two thirds of the anterior lobe. The roentgen rays had been applied to the two temporal fields, the total dosage being approximately 610 roentgens. This observation shows that in the dosage mentioned the roentgen rays may cause marked anatomic changes in the human hypophysis.

MORPHOLOGIC STUDIES OF THE SPERM PICTURE IN THE TESTICLES OF BULLS IN WHICH FERTILITY WAS DECREASED OR LACKING. NILS LAGERLÖF, Acta path. et microbiol. Scandinav., supp. 19, 1934.

This is a noteworthy contribution to the rather meager and indefinite information pertaining to the problem of sterility of bulls. The dissertation consists of 254 pages, including 273 references. The work is generously illustrated, largely by photomicrographs of high technical excellence.

Lagerlöf studied the spermatozoa of approximately one hundred bulls with a record of good fertility and one hundred and fifty bulls in which fertility was decreased or absent. Biometric studies were carried out with regard to the relation of the length of the head of the sperm and the degree of fertility. A study was also made of the pathologic anatomy of the testes of approximately one hundred bulls in which there was anamnestic data of impaired fertility.

The more significant results of the investigations may be stated briefly as follows: 1. The number of spermatozoa in bulls of good fertility varies from 300,000 to 1,200,000 per cubic millimeter of semen; the average being 800,000. 2. The number of abnormal sperms did not exceed 18 per cent, with the average between 10 and 12 per cent. 3. Good motility of sperms may or may not indicate satisfactory fertility. 4. The biometric determination of the length of the head of the sperm yields a variation coefficient not to exceed 4.

In the bull the following may be looked on as indicative of disturbed spermatogenesis with fertility being impaired or absent: (1) If, on repeated examinations, the number of sperms is low (below 200,000 per cubic millimeter); (2) if the

rate of occurrence of pathologic sperms is 20 per cent or higher; (3) if so-called "unripe" spermatozoa are present in large number; (4) if the coefficient of variability of the length of the head of the sperms is 4.4 or above, and (5) if the motility of the sperm on repeated examinations is obviously decreased.

In the limitation of space imposed, an adequate and satisfactory abstract of this monograph is impossible, and those interested in this particular field of comparative pathology should consult this excellent contribution in the original.

WILLIAM H. FELDMAN.

Pathologic Chemistry and Physics

CHANGES IN HEMOGLOBIN IN TISSUE CULTURES. L. DOLJANSKI AND O. KOCH, Virchows Arch. f. path. Anat. **291**:379, 390 and 397, 1933.

In three short communications Doljanski and Koch report the results of investigations undertaken to elucidate the decomposition and synthesis of hemoglobin. Cultures of liver and spleen and pure cultures of fibroblasts were used. In the first communication the formation of methemoglobin is described as determined spectrographically and spectrophotometrically. The use of tissue extract alone resulted in the formation of hematein. In the presence of living cells this action was decreased or inhibited. In the second communication the failure of formation of bilirubin in tissue cultures is recorded. Mixtures of plasma, embryonic extract and Tyrode's solution of hemoglobin, such as were added to the tissue cultures, were incubated without tissue. A formation of bilirubin occurred. They conclude that the formation of bilirubin is a humoral process that does not require the direct activity of living cells. In the third communication the formation in tissue cultures of a yellow pigment, apparently xanthorubin, is described. The formation of methemoglobin reaches its height on the fifth or sixth day of incubation. Methemoglobin then begins to disappear, and the yellow pigment makes its appearance. For the formation of this pigment the activity of living cells is necessary.

O. T. SCHULTZ.

THE DESTRUCTIVE ACTION OF BLOOD SERUM ON HEMOGLOBIN. L. DOLJANSKI AND O. KOCH, Virchows Arch. f. path. Anat. **291**:401, 1933.

When blood serum was added to a freshly prepared solution of hemoglobin in Tyrode's fluid and the mixture incubated, the formation of methemoglobin and later of hematein was noted. Similar changes occur in solutions of hemoglobin not exposed to the action of serum, but in the presence of the latter the process is more rapid and more marked. Human serum had a more pronounced action in this respect than chicken serum or rabbit serum. The authors interpret their findings as evidence of a toxic or destructive action of normal blood serum on hemoglobin. A protective mechanism that prevents action of the serum on the hemoglobin of the corpuscles under normal conditions is postulated.

O. T. SCHULTZ.

CHEMICAL NATURE OF THE ANTIGENS EMPLOYED IN THE DIAGNOSIS OF SYPHILIS. E. BALBI, Ztschr. f. Immunitätsforsch. u. exper. Therap. **78**:524, 1933.

The reacting substances in alcoholic heart extracts were adsorbed with ordinary aluminum hydroxide, while those of brain extracts were not affected by such treatment but were removed by a modified aluminum hydroxide (a product obtained by Willstätter and Schneider). The results were established by means of in vitro and in vivo experiments. The brain-specific antigen adsorbed by the modified aluminum hydroxide can be separated by treatment with benzene.

I. DAVIDSOHN.

HEMOLYSIS, COLLOID STATE AND IRRADIATION. JOHANN SCHUBERT, Ztschr. f. Immunitätsforsch. u. exper. Therap. **80**:205, 1933.

The work is an attempt to throw light on the nature of the hemolytic process by correlating it with the colloid state. Five per cent solutions of the sodium salts of saturated fatty acids were used. By means of the Tyndall phenomenon and the estimation of the surface tension it was found that hemolysis began where colloid systems appeared in molecular solutions. An attempt to change the colloid state by means of ultraviolet radiation succeeded, but the hemolytic function was not affected. The change of the colloid state brought about by the irradiation was not stable, which may explain why hemolysis was not affected.

I. DAVIDSOHN.

THE CHEMICAL NATURE OF BLOOD GROUP FACTORS. E. JORPES AND G. NORLIN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:152, 1934.

The term "blood group factor A" must be understood as meaning more than one chemical substance. The heterophilic fraction which reacts with antisheep hemolysin is combined with polysaccharid substances and is chemically different from the factor which reacts with iso-agglutinins. The latter, as well as the B factor, is thrown down by protein precipitants and is destroyed by enzymes. The two fractions of the blood group qualities A can be separated by precipitation with tannic acid and with salts of heavy metals. The recently reported destructive effect of fecal extracts and of saliva on the blood group factors may be due to digestive ferments.

I. DAVIDSOHN.

INVESTIGATIONS OF POSTOPERATIVE ACIDOSIS AND KETONURIA. FREDRIK ROSCHER, Acta chir. Scandinav., supp. 29, 1933.

Roscher introduces his work with a preliminary discussion of the acid-base balance and biochemistry of the ketone bodies. A series of qualitative studies indicated that a moderate postoperative ketonuria occurs under general and, rarely, under local anesthesia. Roscher made quantitative biochemical studies on a series of seventeen patients subjected to surgical operations under general, local and spinal anesthesia. These studies included determinations of the carbon dioxide content of the blood, the p_H of the venous blood, the nonprotein nitrogen of the blood, and the blood sugar, and quantitative urinary studies including estimations of the total acetone and nitrogen, free acid, ammonia and total base. Finally, experimental studies were made of rabbits with regard to the glycogen content of the liver after anesthesia. Among the significant findings after operation under general anesthesia Roscher notes: a shift to the acid side of the acid-base equilibrium, hyperglycemia, a decrease of glycogen in the liver; an abnormally high nitrogen excretion and excessive urinary excretion of salts, acids and ketone bodies. In children all of these changes were more severe owing to the greater lability of their metabolism. Under local anesthesia these changes were much milder. The hyperglycemia under general anesthesia was demonstrable immediately after the anesthetic began to be administered and before the operation was begun. The blood sugar returned to normal within twenty-four hours after the operation. The postoperative ketosis is ascribed to the profound disturbance of the metabolism under general anesthesia with the inhibition of oxidative processes and disturbances in the metabolism of carbohydrate, protein and fat. Inanition due to fasting and scanty nutrition because of the operation are contributing factors in the development of postoperative ketosis. Roscher gives pertinent data and tables as the basis for his results.

JACOB KLEIN.

THE BLOOD GROUP SPECIFIC PROPERTIES OF URINE. ERIK JORPES, Acta path. et microbiol. Scandinav. **11**:99, 1934.

The blood group specific substances of aqueous solutions of dried concentrates of human urine were precipitated by lead acetate, ammonium sulphate and tannic

acid. A polysaccharid fraction was prepared from the tannic acid precipitate of A-urine which was highly specific in binding the lysin of the antisheep hemolysin which was produced by treating rabbits with human red blood cells of type A. Proteolytic enzymes (papain and trypsin) destroyed the blood group substances of the protein fraction of the urine concentrates. Jorpes concludes that the fraction of blood group substance which binds the iso-agglutinins is protein in nature.

I. DAVIDSOHN.

Microbiology and Parasitology

THE HISTOLOGY OF EQUINE ENCEPHALOMYELITIS. E. W. HURST, J. Exper. Med. **59**:529, 1934.

The virus of equine encephalomyelitis (eastern strain) evokes in the horse, calf, sheep and dog an unusually intense encephalomyelitis characterized by acute primary degeneration of nerve cells, the appearance in neurons of the brain stem and elsewhere of nuclear inclusions resembling those in Borna disease and poliomyelitis, polymorphonuclear infiltration in the nerve tissues with early microglial proliferation, and perivascular cuffing with mononuclears and polymorphonuclears in varying proportions. The gray matter is affected more than the white. Lesions may be less marked in the striatum, brain stem and cord than in the cerebral cortex, thalamus and hypothalamic region, and are always of low grade in the cerebellum. Meningeal infiltration is secondary. Similar changes produced in the horse by the western strain of virus are less intensive and extensive. In the guinea-pig, rabbit and mouse the eastern virus causes an acute encephalomyelitis which, as is usual in neurotropic virus diseases of these lowly species, has a special tendency to affect the higher olfactory centers. In addition to inclusions in the nerve cells, tiny oxyphilic bodies occur with less frequency in the glial and mesodermal nuclei of the guinea-pig. In this animal, too, interstitial pneumonia or bronchopneumonia may complicate the picture. In the guinea-pig the disease resulting from infection with the western virus may be indistinguishable from that due to the eastern.

FROM THE AUTHOR'S SUMMARY.

THE CARRIER PROBLEM IN MENINGOCOCCUS INFECTION. G. RAKE, J. Exper. Med. **59**:553, 1934.

Of the three studies which have been reported in this paper, the most thorough and therefore the most instructive was that made on the Rockefeller Institute group of twenty-four persons. The ten carriers discovered in this group were found to fall into three categories, namely, chronic, intermittent and transient carriers. It is, perhaps, a matter for surprise, in view of the weight of evidence in the literature, that half of the carriers should appear in the chronic group, being constantly affected for periods over two years and continuing to carry throughout this period what was, to all tests, the same strain of micro-organisms. It has been shown that no claim of relief from the carrier condition can be based on three consecutive negative swabs at weekly intervals, since apparent spontaneous "cures," as evidenced by negative swabs, may last for four and one-half months and finally be terminated by the reappearance of the same strain as was carried before. The effect of coryza and pharyngitis on the persistence and degree of the meningococcic infection has been studied and, while the results are scanty, indications have been found that coryza, unassociated with any increase in numbers of the nasopharyngeal pathogens or streptococci, causes no change in the number of meningococci present in the throat. On the other hand, a streptococcic pharyngitis or any infection in which other throat pathogens increase greatly in number is usually associated with a marked diminution or actual disappearance, whether temporary or permanent, of the meningococci from the nasopharynx.

EXPERIMENTAL TYPE III PNEUMOCOCCUS PNEUMONIA IN MONKEYS. T. FRANCIS JR. and E. F. TERRELL, J. Exper. Med. **59**:609, 1934.

It has been possible by the intratracheal or intrabronchial inoculation of Pneumococcus type III to produce in monkeys of the species Macacus cynomolgos an experimental pneumonia which in its clinical aspects closely resembles pneumococcic lobar pneumonia in man. The experimental disease is characterized by the development of a well localized pulmonary lesion of lobar distribution which tends to spread, the frequent occurrence of septicemia, a sustained fever, and the termination of the infection, after a variable interval, in recovery or death of the animal. Wide variations in the severity of the disease in different monkeys have been noted. These variations appear to be due primarily to the differences in the resistance of individual animals. The height of the septicemia accompanying the experimental pneumonia has been found to be the most valuable objective index of the probable outcome of the disease. Other factors which may influence the course and outcome of the disease are discussed.

FROM THE AUTHORS' SUMMARY.

TREATMENT OF TYPE III PNEUMONIA IN MONKEYS WITH ENZYME DECOMPOSING TYPE III CAPSULAR POLYSACCHARIDE. T. FRANCIS JR. and others, J. Exper. Med. **59**:641, 1934.

The effects of specific enzyme therapy on experimental type III pneumococcic pneumonia in monkeys were studied by comparing the course and outcome of the disease in treated animals with those in animals which received no therapeutic aid. Enzyme treatment was followed by cessation of spread of the pneumonic lesion, sterilization of the blood and early recovery, except in animals in which the severity of the disease was extreme. While in the untreated animals a high incidence of empyema and pericarditis was observed, suppurative sequelae were apparently prevented by adequate enzyme therapy. The limitations of the therapeutic action of the specific enzyme in the presence of marked depression of the cellular reaction in infected animals are again emphasized.

FROM THE AUTHORS' SUMMARY.

LOUPING ILL IN MAN. T. M. RIVERS and F. F. SCHWENTKER, J. Exper. Med. **59**:669, 1934.

Four instances of infection in man are described which are believed, because of the circumstances under which they occurred and in view of the results of neutralization tests, to represent cases of louping ill. Evidence obtained in the neutralization tests is in favor of the idea that the antibodies against the virus of louping ill demonstrated in certain serums were most likely the result either of contact with or of infection with the active agent.

FROM THE AUTHORS' SUMMARY.

CYTOLOGY OF THE BLOOD IN EXPERIMENTAL SYPHILIS. P. D. ROSAHN, L. PEARCE and A. E. CASEY, J. Exper. Med. **59**:711 and 721, 1934.

Weekly cytologic observations were made on the blood of seven syphilitic and nine normal control rabbits. Each animal was examined seven times prior to and fifteen times after inoculation of the experimental group. Comparisons were made between the mean blood cell values obtained from all counts on the experimental and control groups in the preinoculation and postinoculation periods. The mean blood cell formula of the syphilitic group for the three and one-half month period after inoculation was significantly different from the preinoculation mean values observed in the same group in the following respects: higher total white cell count, platelet count, neutrophil count and monocyte count, and lower lymphocyte count. The mean blood cell formula of the syphilitic group for the three and one-half month period after inoculation was significantly different from

the mean blood cell formula of the normal control group in the same interval of time in the following respects: higher total white cell count, platelet count, neutrophil count and monocyte count, and lower lymphocyte count. From these results it was concluded that during activity of the disease the blood cytology of rabbits infected with Spirochaeta pallida is characterized by an increase in the total white cell count, the platelet count, and the neutrophil and monocyte counts and a decrease in the lymphocyte count from normal values. These changes were statistically significant.

The mean blood cell levels of thirty-five rabbits with latent syphilis, in which all lesions had undergone spontaneous regression and complete healing, were compared with weighted values for normal rabbits. The only differences noted were in the red cell count and hemoglobin content, both of which in the experimental group were significantly lower than the normal values. A parallelism was observed between the blood cell changes of the experimental disease after spontaneous regression of lesions and the blood cell changes in the human disease after treatment. This parallelism lends additional weight to deductions from the experimental disease as applied to human syphilis.

FROM THE AUTHOR'S SUMMARIES.

PSEUDORABIES (INFECTIOUS, BULBAR PARALYSIS, MAD ITCH). E. W. HURST, J. Exper. Med. **59**:729, 1934.

After intramuscular, intradermal and subcutaneous inoculation, the pseudorabies virus reaches the central nervous system by way of the peripheral nerves, although it is circulating in the blood. Centrifugal spread from the infected nerve tissues by the neural route also occurs. After intracerebral inoculation the virus passes in the reverse direction, down the nerve axis. The Aujeszky strain invades the blood stream more readily than does the Iowa strain; but possibly with repeated passage the latter is approximating in this respect more closely the classic Aujeszky strain. After intravenous inoculation, effective with even small doses, the virus is rapidly removed from the blood, and multiple infective foci are established in various organs; thence ascent of the virus by the peripheral nerves leads to infection of the central nervous system, the symptomatology differing according to whether the spinal cord or the medulla is reached first. The lack of evidence that the virus can penetrate the hemato-encephalic barrier directly deserves emphasis. When subcutaneous inoculation is practiced in an area deprived of its nerve supply, the ability of the virus to invade the blood stream permits it to establish infective foci in the various viscera, and, after a predictable delay, the course of infection resembles that following intravenous injection. The pseudorabies virus is pantropic; i. e., it readily attacks cells derived from any embryonic layer. Lesions in the suprarenal gland following intravenous inoculation are very like those due to herpes virus similarly introduced, this being one point of similarity in the pathogenic action of the two organisms. The relation of the pseudorabies virus to other viruses affecting the central nervous system is discussed.

FROM THE AUTHOR'S SUMMARY.

GROWTH-STIMULATING PROPERTIES OF CYSTINE AND TRYPTOPHAN. W. BURROWS, J. Infect. Dis. **54**:164, 1934.

Cystine and tryptophan, together or separately, exerted a marked growth-stimulating action on strains of Clostridium botulinum when they were included in mediums composed of dextrose and acid hydrolysates of casein or gelatin. The growth-stimulating properties of cystine and tryptophan in these mediums appeared to be qualitatively similar. Of a total of sixteen amino-acids tested, only cystine and tryptophan had this stimulating effect.

FROM THE AUTHOR'S CONCLUSIONS.

PASSAGE OF BOVINE BRUCELLA THROUGH SWINE. H. L. GILMAN, C. H. MILKES and R. R. BIRCH, J. Infect. Dis. **54**:171, 1934.

An attempt was made to ascertain whether passage of bovine strains of Brucella through a series of sows would induce these strains to assume the characteristics of the porcine type, as determined by the dye tolerance tests. One strain was passed through a series of two hogs, two strains through two series of five hogs each, and one strain through a series of six hogs. At no time were we able to detect any change from the bovine to the porcine type, as determined by the reaction of the strains to the presence of basic fuchsin or thionine in the mediums in dilutions of 1:50,000.

FROM THE AUTHORS' SUMMARY.

DEMONSTRATION OF ACID-FAST BACILLI IN TUBERCULOUS FILTRATES. E. L. WALKER and M. A. SWEENEY, J. Infect. Dis. **54**:182, 1934.

The claims of Fontés, Vaudremer, Calmette and many others that the inoculation of filtrates of tuberculous material from supposedly bacteria-proof filters into guinea-pigs may result in an atypical infection have been substantiated. The frequent passage of a few acid-fast bacilli through all the types and grades of filters used by these investigators has been demonstrated by microscopic examination of stained smears of centrifugated precipitates of the filtrates. By inoculation with tuberculous filtrates which were proved microscopically to contain a few tubercle bacilli we produced the atypical nonprogressive infections ascribed to the "ultravirus." The frequent passage of a few tubercle bacilli through bacterial filters would account for all of the phenomena consequent to inoculation of tuberculous filtrates without invoking the existence of Calmette's tuberculous ultravirus. The microscopic demonstration of the passage of tubercle bacilli through supposedly bacteria-proof filters creates presumptive evidence that other bacteria may also pass filters, evidence that will have to be taken into consideration in every claim for filtrable forms of other micro-organisms.

FROM THE AUTHORS' SUMMARY.

SUITABILITY OF HERROLD'S EGG YOLK AGAR MEDIUM FOR THE BOVINE TUBERCLE BACILLUS. W. H. FELDMAN, J. Infect. Dis. **54**:194, 1934.

A study was made to determine the suitability of Herrold's egg yolk agar medium for the isolation and subsequent growth of the organism of bovine tuberculosis. Of seventy-one lymph nodes obtained from cattle which were killed on account of having reacted positively to tuberculin, sixty-three were eventually proved to be tuberculous by inoculation into guinea-pigs. Acid-fast bacillary forms were present in fifty-five of the smears made directly from seventy of the specimens. Emulsions were prepared from the various lymph nodes; a portion of each was treated with 5 per cent oxalic acid for the purpose of controlling contaminants, and cultures were made. The amount of the respective emulsions used for attempts at culture was 0.25 cc.; with eighteen of the specimens, however, additional attempts were made with 1 cc. of the emulsion. Cultures were obtained from forty-three (approximately 68 per cent) of the sixty-three specimens in which cultivable bacteria were assumed to have been present by virtue of the results in guinea-pig controls. Limited observations failed to disclose any significant enhancement of the capabilities of growth of the bovine tubercle bacillus following its passage through guinea-pigs. The cultural requirements of the bovine and of the human form of the organism are dissimilar. A medium that serves satisfactorily for the isolation of the human form may be less acceptable for the bovine form. The egg yolk agar medium of Herrold, while capable of promoting the growth of the bovine tubercle bacillus in a high percentage of instances, cannot be considered an optimal medium for all strains of the organism encountered in spontaneously infected material. The fastidiousness of the organism of bovine tuber-

culosis, when present in spontaneously infected tissues, suggests the use of guinea-pigs rather than of culture mediums in the laboratory examination of clinical material believed to contain this organism.

FROM THE AUTHOR'S SUMMARY AND CONCLUSIONS.

BACTERIAL ACTIVITY IN DIFFERENT LEVELS AND IN ISOLATED SEGMENTS OF THE INTESTINE. G. M. DACK and E. PETRAN, J. Infect. Dis. **54**:204, 1934.

A study of some factors operative in regulating the relationship and maintenance of certain bacteria in the intestine was made on six monkeys, three with nonleaking fistulas into different levels of the intestine, one with a Thiry-Vella fistula and two with isolated segments of colon, and on four dogs with Thiry-Vella and cecal fistulas. Gastric acidity was found not to be a constant barrier to the entrance of organisms into the intestinal tract. No bactericidal substances were observed in the contents of the small intestine, as evidenced by the following: 1. Jejunal contents aspirated from a monkey during digestion proved an excellent medium in which organisms could multiply. 2. Pledgets of cotton dipped in a culture of Bacterium prodigiosum were suspended in the duodenum of one monkey and in the ileum of another. Bact. prodigiosum and other organisms in abundance were obtained from the pledgets removed after eighteen hours. Peristalsis and secretions play an important rôle in rapidly freeing the small intestine of organisms. Support for this view was obtained in the fact that Bact. coli and Bact. prodigiosum rapidly disappeared from Thiry-Vella fistulas into which they had been introduced. The rich fecal flora in the terminal ileum is not due to a difference in the bowel at this level, since the rate of disappearance of Bact. coli and Bact. prodigiosum placed in Thiry-Vella fistulas in dogs was the same regardless of whether the fistulas were of the terminal ileum or of the upper part of the jejunum. The fecal flora in the terminal ileum probably represents regurgitation of cecal contents, as the ileocecal sphincter in a monkey and a dog was not found patent and, furthermore, reverse peristaltic waves were observed in the terminal ileum of another monkey. Isolated segments of colon in two monkeys were found to contain large numbers of Bact. coli and appreciable numbers of streptococci when tested six months following the operation in one case and three months following it in the other. Apparently the colon bacillus is peculiarly adapted to grow and remain viable in the mucous secretions of the empty large bowel.

FROM THE AUTHORS' SUMMARY.

THE FILTRABILITY OF THE ACID-FAST GROUP. F. B. COOPER, J. Infect. Dis. **54**:236, 1934.

In the studies just reported, a white and a chromogenic variant, dissociated from the rapidly growing acid-fast saprophytic Mycobacterium phlei, were used in an attempt to demonstrate the filtrable phase. All cultures contained small rods, beaded forms or single granules at the time of filtration and were considered ideal for such experiments. Growth was never obtained from any filtrate regardless of its age, the type of medium used or the number of serial subinoculations employed. Kendall's medium produced a pleomorphic form which, under the conditions of the experiments, possessed no greater filtrability than similar forms from other unfavorable mediums. Negative results were also obtained from the S variant dissociated from the bovine strain B_1 and from the undissociated human strain H37. The presence or absence of single granules in cultures depends chiefly on the staining technic. A beaded rod may appear as several isolated granules if improperly stained.

FROM THE AUTHOR'S SUMMARY.

RELAPSING FEVER IN CALIFORNIA. G. E. COLEMAN, J. Infect. Dis. **54**:281 and 295, 1934.

Latent infections were not found in the brain, bone marrow or blood of mice that had recovered, or in those of nine gray and ground squirrels and one chipmunk in nature.

A close relationship, as judged by cross-immunity tests, exists between certain California strains of the spirochetes of relapsing fever. The areas from which some of these strains originated are several hundred miles apart. It is believed that by a more accurate management of the technic employed in cross-immunity tests a relationship can be shown to exist between strains which by grosser methods would be considered to have only individual specificity. Serologic evidence has been obtained which favors the probability of a close relationship between certain Texas and California strains of these spirochetes. A study of Sierra golden mantle ground squirrels a month after capture failed to reveal the organism of relapsing fever. All attempts to infect them by several routes, singly or combined, failed. Their blood serum, both before and after the injection of several strains, failed to protect mice not only against the strains injected into the squirrels but in some instances against other strains as well. It seems probable that this species is immune to relapsing fever.

FROM THE AUTHOR'S SUMMARIES.

UNDULANT FEVER IN NEW YORK STATE. R. GILBERT and M. B. COLEMAN, J. Infect. Dis. **54**:305, 1934.

If micro-organisms of the abortus-melitensis group can be divided into subspecies or types, our findings indicate that only one—the bovine type—is prevalent in the relatively large area served. Furthermore, the information available suggests that cattle or dairy products have been the source of the infection in most of the cases of undulant fever studied. A large percentage of the patients have had no contact with cattle, but have used raw milk or cream. A marked decrease in the incidence of undulant fever in New York State might be expected in the event of general, efficient pasteurization.

FROM THE AUTHORS' SUMMARY.

ACID-FAST ORGANISM FROM LEPROUS LESION IN TISSUE CULTURES AND OTHER MEDIUMS. A. J. SALLE, J. Infect. Dis. **54**:347, 1934.

Chick tissue cultures, prepared according to the method of Carrel, were used as a culture medium for the isolation of the organisms of human and rat leprosy. An acid-fast organism and a diphtheroid were isolated from four human nodules and one rat granuloma. When transfers were made to artificial culture mediums (egg, potato, agar, etc.) only the blue-staining diphtheroid multiplied. After the primary isolations on chick tissue cultures, minced chick embryo medium furnished an excellent substrate for the cultivation of the organisms. As with chick tissue cultures, the minced embryo medium gave rise to acid-fast and nonacid-fast organisms. A pure culture of the diphtheroid when inoculated into chick embryo medium gave acid-fast and nonacid-fast organisms. The diphtheroid and the acid-fast rods are apparently different phases of growth of the same organism. The organisms were acid-fast in tissues and nonacid-fast on laboratory mediums. The tinctorial characteristics varied, depending on the living condition of the tissues. In vigorous, actively growing tissue the organisms were strongly acid-fast. As the tissue became less vigorous the acid-fast property was less pronounced and, finally, as the tissue died, only nonacid-fast diphtheroids were seen. It is believed that human and rat leprosy are caused by one and the same organism.

FROM THE AUTHOR'S SUMMARY.

POSTMORTEM BACTERIOLOGY. C. G. BURNS, J. Infect. Dis. **54**:388 and 395, 1934.

Clostridium Welchii, Bacterium coli and staphylococci are capable of invading the tissues of animals within from five to forty-eight hours after death when the bodies are kept at 25 C. A large group of pathogenic and nonpathogenic bacteria failed to invade the tissues after death, even though ample opportunity was given for invasion to occur. The quantity of organisms, their location within the body and the time between death and autopsy are undoubtedly important factors influenc-

ing postmortem invasion. A temperature of 10 C. inhibits the growth of Bact. coli and Cl. Welchii even after ninety-six hours' incubation. Increased temperature failed to bring about the spread of strains of bacteria known to be noninvasive.

The organs and body fluids show a high incidence of bacterial growth at necropsy. The lungs, kidneys, liver, spleen and cardiac blood show frequency of growth in the order named. Cultures of blood from the heart do not present the true bacterial flora of the human body at necropsy. Bact. coli, staphylococci, Streptococcus nonhaemolyticus and Cl. Welchii are the most frequently isolated, while the other types isolated are usually associated with disease processes within the body. No significant differences can be demonstrated in either the frequency or the kinds of bacteria isolated from the organs post mortem between the first and forty-eighth hour after death. No information is available pertaining to bacterial invasion during the first hour after death or at the time of death. All the bodies studied were subjected to a temperature of 10 C. within a short time after death and were kept in this environment until the autopsy was performed.

FROM THE AUTHOR'S SUMMARY AND CONCLUSIONS.

THE NATURE AND ANTIGENIC PROPERTIES OF A HIGHLY PURIFIED PHAGE. I. J. KLIGLER and L. OLITZKI, Brit. J. Exper. Path. **15**:14, 1934.

Kaolin adsorption of phage from broth cultures followed by elution with hundredth-normal ammonium hydroxide yields a potent phage relatively free from protein. Successive elutions from the same kaolin adsorbate followed by dialysis yield active phage suspensions giving negative protein and ninhydrin reactions. The residue on the kaolin after repeated elutions has a marked inhibitive effect on the phage. This effect does not appear to be specific. The purified phage retains its antigenic potency.

FROM THE AUTHORS' CONCLUSIONS.

THE PROPAGATION OF THE VIRUS OF INFECTIOUS LARYNGO-TRACHEITIS ON THE CHORIO-ALLANTOIC MEMBRANE. F. M. BURNET, Brit. J. Exper. Path. **15**:52, 1934.

The virus of laryngotracheitis may be propagated in the chorio-allantoic membrane of the developing egg. The lesions produced in the membrane are primarily due to proliferative and necrotic changes in the ectodermal layer; the proliferating cells frequently show typical intranuclear inclusions similar to those found in the tracheal lesions.

FROM THE AUTHOR'S SUMMARY.

PROPAGATION OF THE VIRUSES OF FOWL PLAGUE AND NEWCASTLE DISEASES ON CHORIO-ALLANTOIC MEMBRANE. F. M. BURNET and J. D. FERRY, Brit. J. Exper. Path. **15**:56, 1934.

The viruses of Newcastle disease and fowl plague are highly infective for the developing egg, and the use of the related technic for their investigation offers a number of advantages. The virus of Newcastle disease produces a characteristic lesion in the chorio-allantoic membrane, in which cytoplasmic inclusions can be demonstrated histologically. Comparative filtration studies with egg material indicate that the virus is larger (from 80 to 120 millimicrons) than the virus of fowl plague (from 60 to 90 millimicrons). The virus of Newcastle disease is more resistant to photodynamic inactivation by methylthionine chloride U. S. P. (methylene blue) than the virus of fowl plague. These differences, in conjunction with the known clinical and immunologic differences, point to the complete etiologic independence of the two diseases.

FROM THE AUTHORS' SUMMARY.

Immunology

THE BACTERICIDAL PROPERTIES OF LEUCOCYTES AND BLOOD-PLATELETS IN THE PRESENCE OF NORMAL SERUM. T. J. MACKIE, C. E. VAN ROOYEN AND M. H. FINKELSTEIN, J. Path. & Bact. **39**:89, 1934.

The bactericidal action of serum-leukocyte preparations is dependent on (*a*) the bactericidal action of the normal serum per se, (*b*) the bactericidal action of the leukocytes per se and (*c*) an enhanced bactericidal effect quantitatively in excess of the sum of these properties (*a*) and (*b*); serum-leukocyte mixtures may also yield a bactericidal reaction even when the serum and leukocytes are apparently inactive per se. The bactericidal action of leukocytes per se and the enhanced action of serum plus leukocytes occur in the absence of any phagocytosis of the bacteria, e. g., (*a*) after the cells have been heated at 45 C., or higher temperatures in the case of rabbit leukocytes, (*b*) after treatment with a leukocidal toxin. The bactericidal properties depend on an extractable product of the cells, and the enhanced bactericidal effect is a function of the mutual interaction of this product and a serum principle; this leukocyte substance is liberated under the conditions of the bactericidal experiment, though it may possibly act intracellularly after the bacteria have been sensitized by the serum and phagocytosed. Blood platelets, like leukocytes, may exhibit bactericidal action per se and an enhanced effect in the presence of serum; their action also depends on an extractable substance which is similar in its properties to that of the leukocytes. Treatment of leukocytes with a leukocidal toxin may increase their bactericidal activity both per se and when tested with serum, probably by increased liberation of this active substance. The serum principle which is mutually active with leukocytes and platelets is thermostable up to 63 C. The active substance of the leukocytes and platelets is exceedingly labile in the ox and horse (i. e., at 50 C.) but that of rabbit leukocytes is more stable, remaining active at 55 and even 60 C. More than one leukocyte or platelet substance of this type may be concerned in these reactions; thus the substance reacting with certain gram-negative bacteria is less easily extractable from the cells than that which affects the gram-positive types. The mutally active principles of serum and leukocytes (or platelets) cannot be identified with the B lysin or a component of this lysin. The enhanced effect referred to may be absent when the activity of the serum on the one hand or of the leukocytes (or platelets) on the other exceeds a certain limit, and the combined bactericidal effect of serum plus leukocytes (or platelets) may in such cases be less than the independent effects of the serum and cells. The enhancement depends on a suitable proportion of the active principles concerned.

FROM THE AUTHORS' SUMMARY AND CONCLUSIONS.

LOCAL EFFECTS OF THE INTRA-ARTICULAR INJECTION OF LACTIC ACID IN SENSITIZED RABBITS. A. VANNOTTI, Virchows Arch. f. path. Anat. **292**:55, 1934.

Rabbits were sensitized by the subcutaneous injection of horse serum on alternate days during a period of eight days. During this period two injections of a mixture of lactic acid and lithium carbonate were made into a knee joint, the contralateral joint serving as a control. Three weeks after sensitization the animals received 3 cc. of horse serum intraperitoneally and another injection of the lactic acid solution into the joint. The animals were killed at intervals and the joints subjected to examination. The reaction of the lactic acid-lithium carbonate solution varied from p_H 4.5 to 7.5. The inflammatory reaction invoked in the joint receiving the injection was much more intense in the sensitized animals than in the normal controls. In the former the more acid solutions caused the greater degree of reaction. The inflammatory reaction is held to be allergic. It is the result of a local fixation of antigen brought about by the action of the lactic acid solution on the joint tissues. The mechanism of antigen fixation is not understood; it may

be the result of greater capillary permeability caused by the lactic acid. The antigen fixation does not appear to be the result of the hydrogen ion concentration of the lactic acid solution, but of the molar concentration of the latter.

O. T. SCHULTZ.

ADMISSIBILITY OF BLOOD TRANSFUSION IN AUTO-AGGLUTINATION. O. LEWIN, Wien. klin. Wchnschr. **47**:715, 1934.

Autohemagglutination is an extremely rare phenomenon which has been observed twice in a series of 3,000 blood group determinations. Lewin discusses the question of blood transfusion in such cases and decides that it is permissible provided the blood grouping is determined with washed erythrocytes of the patient. The same blood group must be used in the transfusion. He reports his experience with a man 40 years old who suffered from an obscure hepatosplenic disorder which required splenectomy. The patient received a blood transfusion (500 cc., group O) the day before the operation with no reaction whatever to the transfusion. The patient died five hours after operation as the result of shock and hemorrhage. Autopsy twenty minutes after death revealed hepatic cirrhosis, intraperitoneal hemorrhage and agglutination. It was decided that the latter had occurred after death and was due to cold. Auto-agglutination rarely if ever occurs above 25 C.

THE DISTRIBUTION OF THE BRAIN ANTIGEN IN THE NERVOUS SYSTEM. F. PLAUT, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:46, 1933-1934.

The presence of the organ-specific brain antigen was established in the gray and white matter of the brain, in the gray matter of the cerebellum, in the spinal cord and its roots, in the sympathicus and vagus, in the pineal gland and in the posterior lobe of the hypophysis. It was absent in the anterior lobe of the hypophysis. With the exception of the posterior lobe of the hypophysis, where the quantity of the antigen was somewhat less than elsewhere, there were no quantitative differences.

I. DAVIDSOHN.

IMMUNIZATION WITH ADSORBED HAPTENS WITHOUT PROTEIN. F. PLAUT and H. RUDY, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:87, 1933-1934.

Rabbits inoculated with mixtures of alcoholic extracts of ox brain with kaolin, aluminum hydroxide or soluble starch produced organ-specific immune serums. The results confirm similar previous reports. The response was less regular than when foreign protein was employed to complement the lipoidal haptens. Addition of the aforementioned adsorbing mediums did not enable cholesterol and alcoholic extracts of Spirochaeta pallida to produce specific antiserums when the mixtures were injected into rabbits.

I. DAVIDSOHN.

COMPLEMENT-FIXING ANTIBODIES IN CHICKEN LEUKOSIS OR ERYTHROBLASTOSIS. O. THOMSEN, J. ENGELBRETH-HOLM and A. ROTHE MEYER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:121, 1933-1934.

About 20 per cent of the serums of chickens with experimental leukosis or erythroblastosis gave positive complement fixation with an antigen prepared from the immature blood cells. That the reaction was due to a development of isoantibodies against the injected blood cells and not to antibodies against the introduced virus became apparent from the following observations: 1. The serums of chickens which were inoculated with blood cells of other normal chickens fixed complement quite as well as the serums of infected chickens. 2. The complement-fixing antibodies in the serums of chickens which were inoculated with leukosis and of those which were treated with normal blood cells were readily absorbed with normal red blood cells. Inoculation of chickens with red blood cells of other normal chickens did not increase their resistance against subsequent inoculation with the virus of leukosis.

I. DAVIDSOHN.

THE TOXON OF THE DIPHTHERIA TOXIN. R. PRIGGE, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:185, 1933-1934.

Injections of not fully neutralized but not fatal mixtures of toxin with antitoxin are known to produce frequent paralyses in guinea-pigs. Ehrlich explained this by postulating a less toxic product which he called toxon in addition to the more poisonous toxin. His dualistic hypothesis was opposed by another of Arrhenins and Madsen, who explained the phenomenon on a purely chemical basis by comparing the processes in a mixture of toxin and antitoxin with the reactions which take place in mixtures of weak acids and bases. Neither of the two explanations was fully satisfactory. Prigge reviews the whole problem and describes five different forms of intoxication with diphtheria toxin in guinea-pigs. A reduction of the quantity of toxin increased somewhat but very irregularly the number of paralyses. Repeated injections of very small doses of toxin (for instance, $20 \times \frac{1}{40}$ of the lethal dose) led with great regularity to the development of paralyses, while chronic marasmus was rare. The action of incompletely neutralized but not fatal mixtures of toxin and antitoxin is explained by Prigge as being due to continuous separation of small fractions of free toxin over a long period. There is no need to assume more than one toxic substance in the diphtheria toxin.

I. DAVIDSOHN.

THE TOXIN OF THE SCARLET FEVER STREPTOCOCCUS. N. N. SPASSKY, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:336, 1933-1934.

In rabbits the toxin had a marked constricting effect on the blood vessels of the isolated ear as measured by the number of the drops of the dilution of toxin which passed through the blood vessel in comparison with the rapidity of the flow of Ringer's solution. The degree of constriction was proportionate to the concentration of the toxin, but the effect of the same toxin was different in the ears of different rabbits. That is explained by the individual susceptibility of different animals. The toxin did not affect the growth of, and the consumption of carbohydrates in, tissue cultures of hearts of chicken embryo, of the renal cortex of rabbits and of human embryonal mesenchyma. Cultures of Paramecium caudatum, Glaucoma scintillans Ehrenbergi and Euglena gracilis were not killed by the toxin, which is contrary to the findings of Tunnicliff.

I. DAVIDSOHN.

THE CONCENTRATION OF SODIUM CHLORIDE AND THE AGGLUTINATION OF STROMA OF RED CELLS. F. OTTENSOOSER and A. LENZINGER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:354, 1933-1934.

The inhibition of iso-agglutination in hypertonic solutions of sodium chloride is, according to Ottensooser and Lenzinger, due to two factors: (*a*) an interference with the binding of iso-agglutinins and (*b*) a damage to the agglutinable fraction of the agglutinogen. The latter factor was evidenced by the observation that some of the inhibition remained even after the isotonicity was restored before the iso-agglutinating serum was added. Ottensooser and Lenzinger distinguish in the stroma of red blood cells agglutinable and binding substances. They also postulate the presence of an activating substance, the colloidal state of which determines the agglutinability. The agglutinability of stromata is reduced by the hypotonicity which was necessary for their production. In hypertonic solutions the loss of agglutinability of stromata is less marked than of red blood cells; it becomes more marked when isotonicity is restored. When a physiologic solution of sodium chloride is added to a solution of red blood cells in water a turbidity results which is dependent on the rapidity with which the lysis of the red blood cells is brought about. The slower that process takes place, the more intensive the turbidity. The agglutinability of the stromata is directly proportionate to the turbidity.

I. DAVIDSOHN.

THE FLOCCULATION REACTION OF HENRY FOR THE DIAGNOSIS OF MALARIA. W. VOIGTLAENDER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:377, 1933-1934.

The test is based on an antigen-antibody reaction. The antibodies which develop in malaria are, according to Henry, so-called anti-endogens, or antibodies against the endogens or antigenic substances produced in the body proper. These endogens are the iron-containing pigment and melanin. The artificially prepared antigens for the test contain iron salts and melanin. The melanin-containing antigen is the better of the two. The test was found positive in seventeen patients with syphilis of the central nervous system who had been inoculated with malaria. It became positive as a rule only after the second attack and remained positive for a considerable period after the disease was terminated by proper therapy. The test was negative in a large number of different controls. It was positive in acute, latent and chronic forms of avian malaria. In infections of animals which are related to malaria (piroplasmosis and halteridium infections) the test was negative.

I. DAVIDSOHN.

THE TECHNIC OF IMMUNIZATION ACCORDING TO FRIEDBERGER AND OSHIKAWA. S. BELÁK and J. PÁTER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**: 401, 1933-1934.

Friedberger and Oshikawa claimed that immunization may take place without the entrance of antigen into the circulation. They injected bacteria intracutaneously into the ear and amputated the ear after ten minutes. In their opinion the antibody response was due to a distant action of the antigen, because it was assumed that the antigen was removed with the amputated organ and that it did not get in contact with the antibody-producing tissue. That hypothesis is made invalid by the present report of Belák and Páter, who found that the amputation of the ears of rabbits from three to fifteen seconds after intracutaneous injections of from 0.1 to 0.015 cc. of suspensions of paratyphoid B bacilli did not prevent the bacteria from spreading into the general circulation.

I. DAVIDSOHN.

THE EFFECT OF DIGITALIS ON THE ABILITY OF RABBITS TO PRODUCE AGGLUTININS. J. PÁTER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:403, 1933-1934.

From previous investigation it was known that stimulation of the parasympathetic nervous system enhances the antibody response to simultaneously injected antigens. Digitalis was supposed to have such a stimulating effect on the parasympathetic nervous system. Páter could not find this pharmacologic effect of the drug, and in agreement with that finding in rabbits which were given digitalis during immunization with partyphoid B bacilli the agglutinin response was less good than in control animals. The uniformly weaker response may be due to intoxication.

I. DAVIDSOHN.

THE EPIDEMIOLOGICAL SIGNIFICANCE OF THE WEIL-FELIX REACTION IN TYPHUS FEVER. M. P. ISABOLINSKI, R. M. SOBOLEWA, N. J. STRATANOWITSCH, S. L. RIWKINA and T. A. MOSKALEWA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:405, 1933-1934.

The reaction was positive in about 7 per cent of healthy physicians, nurses and hospital personnel who took care of patients with typhus but who never themselves contracted the disease. The reaction remained positive for about one to one and one-half years after recovery from the disease. Its epidemiological significance for the study of carriers is not great partly because frequent positive Widal reactions were observed in the same persons. The deciding test is the production of a characteristic febrile reaction in guinea-pigs into which the blood of patients or carriers has been injected.

I. DAVIDSOHN.

SEROLOGIC DIFFERENTIATION BETWEEN GRAY AND WHITE MATTER OF THE CENTRAL NERVOUS SYSTEM. H. REICHNER and E. WITEBSKY, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:410, 1933-1934.

Immune serums produced by inoculation of rabbits with boiled gray matter of the brain or with white matter of the brain and peripheral nerves reacted specifically with the homologous antigens, thus permitting the serologic differentiation of the two complements of the nervous system. In lower dilutions, these serums reacted with brain tissue in general, behaving like organ-specific immune serums. In all dilutions there was no evidence of species specificity. Immune serums produced with cerebellum, medulla oblongata and spinal cord reacted strongly with extracts of white matter and only moderately with those of gray matter. Antiserums against white matter of the brain reacted with alcoholic extracts of fish brains but not with those of embryonal brains of mammals which lack the myelin substances, while antiserums against the gray matter reacted with extracts of embryonal mammalian brain but not with extracts of brains of various fishes which lack the corresponding gray matter.

I. DAVIDSOHN.

EFFECT OF VITAMINS AND OF ULTRAVIOLET RAYS ON DIPHTHERITIC IMMUNITY OF GUINEA-PIGS. CHIN KUK CHOUN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:432, 1933-1934.

The immunity was tested with intracutaneous injections of diluted diphtheria toxin after a preliminary treatment with vitamin-rich food or with ultraviolet rays or with both. The vitamins proved more potent in raising the immunity than the irradiation; in fact, the latter seemed to weaken the effect of the former, although irradiation by itself had a distinctly immunity-raising effect. Animals kept in rooms with good daylight and fed vitamin-rich food displayed a markedly increased immunity, while the influence of the same food was less potent when the animals were kept in the dark. On the other hand, no such difference in diphtheritic immunity was observed between animals kept in the dark and those kept in daylight when the regular diet was administered.

I. DAVIDSOHN.

THE DROP OF THE TITER OF THE COMPLEMENT IN HISTAMINE SHOCK. B. PAUL AND H. POPPER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **82**:25, 1934.

The well known decrease of complement during anaphylactic shock can be explained either by its fixation in the course of the antigen-antibody reaction or by a change of the colloidal balance of the blood. The histamine shock resembles very closely the anaphylactic shock. Paul and Popper found a marked drop in the titer of the complement in guinea-pigs and dogs which had been given injections of proper doses of histamine. As no antigen-antibody reaction can occur in histamine shock, the drop of complement cannot be attributed to such a phenomenon. In rabbits shocked with histamine the titer of the complement was not affected. The symptoms of the anaphylactic shock are known to be absent or greatly lessened in the animal whose reticulo-endothelial system is blocked. Guinea-pigs and dogs into which india ink had been injected succumbed to shock after injection of large doses of histamine, but the complement was not lowered. Paul and Popper conclude that an intact reticulo-endothelial spleen is necessary for lowering of the complement. The rôle of the liver in the maintenance or lowering of the complement is based on the presence in the liver of a large part of the reticulo-endothelial system.

I. DAVIDSOHN.

THE INHIBITION OF NORMAL HEMOLYSIS IN TYPICAL HEMOLYTIC SYSTEMS. IVAN GYOERFFY, Ztschr. f. Immunitätsforsch. u. exper. Therap. **82**:39, 1934.

When sheep red corpuscles were added to active ox serum, only very faint lysis took place, but inactivated ox serum plus guinea-pig complement laked sheep blood very intensively. In contrast to this, the red corpuscles of the guinea-pig

were readily laked by active ox serum. In the first example, the ox serum exhibited strong, natural, sensitizing but weak complementary properties for the red corpuscles of the sheep. In the second example, both properties were adequate. That indicates differences in the complementary properties of a serum with regard to red corpuscles of different species. Gyoerffy believes that, in addition to dissimilarity in the complement, the slowness of the lysis of sheep cells is responsible for the different behavior of the ox serum. The lysis of a small number of sheep red corpuscles liberates an enzyme which brings about coagulation of the residual fibrinogen of the ox serum. The fibrinogen rapidly envelops the bulk of the unlaked red corpuscles and prevents further lysis. That protection is sufficiently potent to inhibit the action of even distilled water and chemical agents such as chloroform and ether. On the other hand, the rapid lysis of guinea-pig corpuscles takes place before the liberated enzyme brings about coagulation of the fibrinogen.

I. DAVIDSOHN.

THE BLOOD ANTIGENS OF THE SHIGA DYSENTERY BACILLUS. F. SCHIFF, Ztschr. f. Immunitätsforsch. u. exper. Therap. **82**:46, 1934.

The Shiga dysentery bacilli are known to contain, in addition to the heterophilic (Forssman) antigen, two antigenic substances in common with human red corpuscles. One of them is the species-specific human blood antigen; the other is a group-specific antigen related to or identical with the human A_2 factor (Landsteiner) and with the 0 factor (Schiff). Schiff employed an immune serum which was produced in a goat by injections of Shiga dysentery bacilli. The serum agglutinated only weakly all human red corpuscles, but those of group 0 and of subgroup A_2 were agglutinated selectively in high dilutions. There are natural variants of Shiga dysentery bacilli which lack the heterophilic and the human blood antigens. Similar results can be obtained artificially by adding bacteriophage to the culture medium on which the Shiga bacilli are grown. The bacteriophage deprives them of the heterophilic as well as of the human antigens. In view of the fact that ordinary culture medium contains peptone, which is antigenically related to the human blood group factor A, Schiff investigated the possibility that the Shiga bacilli get their human blood antigenic substances from their food. He grew them on a synthetic medium free from constituents of animal origin. The antigenic properties of the bacilli remained unchanged, proving that their origin is not dependent on the constitution of the culture medium.

I. DAVIDSOHN.

INCREASE OF SPECIFIC SENSITIVENESS OF SEROLOGIC REACTIONS. T. T. SCHREUS and R. FOERSTER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **82**:53 and 59, 1934.

An attempt to increase the sensitiveness of the complement-fixation test for syphilis without sacrificing its specificity is described. In a preliminary titration a syphilitic serum known to be strongly positive is tested to determine the smallest amount which is able to fix complement in the presence of one of the usual lipoidal antigens. A quantity of positive serum which is smaller than the fixing dose is added to the unknown serum in the main test. Proper controls are set up to make sure that the added serum is unable by itself to fix complement. The procedure is expected, by the addition of specific reagins, to reveal small quantities of syphilitic complement-fixing reagins that heretofore were below the threshold of sensitivity. To insure a greater constancy of the positive serum, a properly prepared solution of its globulin precipitate is used. The method was tried in comparative tests with one thousand serums. Schreus and Foerster are satisfied that the results have lived up to their expectations. The different flocculation reactions (Kahn, Mueller ball reaction and Meinicke clearing reaction) were distinctly more sensitive, with about 1.2 per cent of false positive reactions. The reaction of Schreus and Foerster was falsely positive in about 1.5 per cent of the

tests. In the course of treatment, the regular Wassermann reaction was the first to turn negative, followed by the reaction of Schreus and Foerster. The precipitation reactions displayed their well known tendency to remain positive.

I. DAVIDSOHN.

THE ORGAN SPECIFICITY OF THE AUTONOMOUS NERVOUS SYSTEM. F. PLAUT, Ztschr. f. Immunitätsforsch. u. exper. Therap. **82**:65, 1934.

Plaut reported previously that the organ-specific cerebral antigen is also represented in the peripheral cerebrospinal and autonomous nerves. The present report is concerned with attempts to differentiate from that general neurohapten special antigenic substances specific for the peripheral and especially for the autonomous nerves. The usual procedure of inoculating rabbits with suspensions of sympathetic and vagus nerves and testing such immune serums against alcoholic extracts of various parts of the nervous system did not reveal any differences. However, when the alcoholic extracts were first evaporated and then resuspended in physiologic solution of sodium chloride, such suspensions of the sympathetic and vagus nerves lost their ability to fix complement, while suspensions of the cerebral cortex had the usual potency, and sciatic nerve tissue was midway between the two extremes. The opalescence of the evaporated alcoholic extracts after suspension in physiologic solution of sodium chloride was more pronounced in the case of the active cerebral cortical tissue than in the others. Suspensions of boiled tissue permitted a sharp differentiation in the complement-fixation test of the cerebral cortical tissue from the sympathetic, vagus and sciatic nerves. An attempt to distinguish serologically the medulla of the suprarenal gland from other parts of the nervous system failed.

I. DAVIDSOHN.

THE GROUP-SPECIFIC PROPERTIES OF ANTI-A IMMUNE SERUM. HERMANN EYER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **82**:74, 1934.

Human red corpuscles of group A contain (among others) the group-specific and the heterophilic (Forssman) antigenic fractions. There has been a discussion going on in the literature concerning the question whether the immune serums produced by inoculation of rabbits with group A corpuscles contain antibodies against both the aforementioned antigens or only against the Forssman antigen. Eyer absorbed such immune serums with sheep red corpuscles and found that their ability to fix complement with extracts and with watery solutions of corpuscles A remained quantitatively preserved after complete absorption of heterophilic antibodies (those against sheep cells and guinea-pig organs). The results confirm previous reports by Witebsky.

I. DAVIDSOHN.

AN ANALYSIS OF AGGLUTININS AGAINST BACILLUS TYPHOSUS. GUELMINO, Ztschr. f. Immunitätsforsch. u. exper. Therap. **82**:82, 1934.

Human immune agglutinins for the typhoid bacillus are known to contain a thermostable fraction (beta-agglutinin or agglutinoid) which is bound by the bacillus without visible agglutination. When such sensitized bacilli are subsequently placed in unheated human antityphoid serum they fail to become agglutinated. However, they do become agglutinated when mixed with a rabbit antityphoid immune serum. Guelmino found that paratyphoid A and B bacilli behaved like typhoid bacilli when treated with human typhoid immune serum, but when they were subsequently placed in rabbit immune serum some strains became agglutinated and others did not. Guelmino explains this phenomenon by the hypothesis that the thermostable haptophore group of the typhoid agglutinins is able to react nonspecifically with numbers of the typhoid-paratyphoid group, but that it makes such sensitized bacteria only specifically agglutinable by the agglutinophore group of the agglutinins of a different animal species.

I. DAVIDSOHN.

Tumors

ADAMANTINOMA OR AMELOBLASTOMA OF THE HYPOPHYSEAL DUCT REGION. M. OLIVER and E. SCOTT, Am. J. Cancer **21**:501, 1934.

Squamous epithelial rests in the region of the infundibulum and capsule of the anterior lobe are commonly regarded as derived from remnants of the embryonic hypophyseal duct of the pars buccalis. Origin from cells of the pars tuberalis has been suggested. Fifty verified cases of adamantinoma of the hypophyseal region and three probable cases lacking histologic verification were collected from the literature. Most of the patients presented Fröhlich's syndrome; none had a history of acromegaly; most displayed atrophy of the optic nerve and disturbances of the eye muscles. Symptoms of intracranial pressure were common. The completeness of this clinical picture was correlated with the age and the extent of the injury done by the tumor to the hypophysis and the neighboring brain structures. The majority of the cases occurred in adolescence or in early adult life. A histologically verified case of adamantinoma of the hypophyseal duct region is reported.

FROM THE AUTHORS' CONCLUSIONS.

THE METASTASIS OF CARCINOMA TO THE SPLEEN. S. WARREN and A. H. DAVIS, Am. J. Cancer **21**:517, 1934.

On the basis of frequency of metastasis, splenic tissue in man has no antineoplastic properties. The paucity of lymphatics and the motility of the organ adequately explain the rarity of metastasis.

FROM THE AUTHORS' CONCLUSION.

IRRITATION VERSUS GENETIC CONSTITUTION IN THE ETIOLOGY OF MALIGNANT TUMORS. M. R. CURTIS, W. F. DUNNING and F. D. BULLOCK, Am. J. Cancer **21**:554, 1934.

The percentage of cysticercus cysts which became malignant increased directly with the age of the enclosed parasites. The percentage of cysts of the same age which became malignant did not differ significantly in male and female hosts. There was an inverse relationship between the number of cysts per host and the proportion of cysts which became malignant. The proportion of tumor cysts among cysts of the same age showed more consistently significant differences when the population was divided according to the number of cysticercus cysts per host than when it was separated according to the strains of the hosts or the cysticercus tumor history of the parents of the hosts. Cysts from hosts with a single cyst showed a significantly higher percentage of malignant changes than cysts of the same age from the general population or hosts with five cysts each or hosts both of whose parents had cysticercus sarcoma.

FROM THE AUTHORS' SUMMARY.

THE MALIGNANT CELLS OF CROCKER CYSTICERCUS SARCOMA. W. MENDELSOHN, Am. J. Cancer **21**:571, 1934.

The malignant cells of two cysticercus spindle cell sarcomas, IRS 4337 and IRS 4338, have been identified. The tumor cells continued to be characteristic of the growth in chicken plasma of explants prepared from tumors transplanted into other rats as well as from the primary tumors. The tumor cells of IRS 4337 and IRS 4338 differ morphologically from the normal fibroblast; they are larger; the cytoplasm is more granular and dense; the nuclei are larger and coarser, and the chromosome number is more varied than that of the normal cell. The tumor cells and nuclei of IRS 4338 are larger than the tumor cells and nuclei of IRS 4337. In the stained preparations the nuclei of IRS 4338 appear to be more

granular than those of IRS 4337. The malignant cells of IRS 4337 and 4338 originated from modified normal cells residing in the walls of the liver cysts; in tissue cultures they can be distinguished by their cytologic characteristics from normal cells.

FROM THE AUTHOR'S RÉSUMÉ.

RED PIGMENTED TUMOR (ERYTHROPHOROMA) IN A FLATFISH. G. M. SMITH, Am. J. Cancer **21**:596, 1934.

A cutaneous red-pigmented tumor (erythrophoroma) with widespread secondary growths is described, occurring in a flatfish (Pseudopleuronectes americanus) from the waters of Long Island Sound.

FROM THE AUTHOR'S SUMMARY.

ADENOCARCINOMA OF THE UTERUS IN A RABBIT. O. I. CULTER, Am. J. Cancer **21**:600, 1934.

A case of adenocarcinoma of the uterus of a rabbit is described. The literature relating to spontaneous tumors of the rabbit is briefly reviewed. Adenocarcinoma of the uterus is the neoplasm most frequently seen in this animal.

FROM THE AUTHOR'S CONCLUSIONS.

BODY TEMPERATURE AND TUMOR GROWTH. W. H. WOGLOM, Am. J. Cancer **21**: 604, 1934.

Raising the body temperature several degrees even for a total period of 348 hours had no adverse effect on the growth of the transplantable tumors employed.

FROM THE AUTHOR'S SUMMARY.

MEGAKARYOCYTOSIS IN WHITE MICE WITH SPONTANEOUS MAMMARY CARCINOMA. W. C. HUEPER, Am. J. M. Sc. **188**:41, 1934.

In addition to extramedullary megakaryocytes of embolic origin (lung, kidney), autochthonous megakaryocytes also occur in various organs (spleen, liver, lymph nodes) of white mice with spontaneous mammary carcinoma. This is supported by the following circumstantial and direct histologic evidence: (*a*) The distribution of megakaryocytes in the various organs precludes their exclusively embolic origin from the bone marrow. (*b*) The absence or relative rarity of traumatized megakaryocytes in the spleen, liver and lymph nodes refutes the assumption that these cells have been carried to these organs by way of the blood vessels. (*c*) The relations existing between the frequency and distribution of heterotopic megakaryocytes and the same observations of extramedullary myeloid foci support the probability of the local origin of heterotopic giant cells. Proliferative foci in the form of megakaryocytic clusters in the spleen and transitional cell forms between reticulum and reticulo-endothelial cells and megakaryocytes in the spleen, lymph nodes and liver point to the local genesis of these cells.

FROM THE AUTHOR'S SUMMARY.

TUMORS AND TUMOR-LIKE CONDITIONS OF THE LYMPHOCYTE, THE MYELOCYTE, THE ERYTHROCYTE AND THE RETICULUM CELL. G. R. GALLENDER, Am. J. Path. **10**:443, 1934.

A discussion of the tumors and tumor-like conditions arising from the stem cells of the lymphocytes, the granular leukocytes, the red blood corpuscles and the reticulum cells or monocytes is presented. A classification of these conditions is presented, based on a study of the cases of the Lymphatic Tumor Registry of the American Association of Pathologists and Bacteriologists. Certain criteria for the differentiation of the conditions are given in explanation or elaboration of the tabular presentation of the classification. Certain evidence is presented that

some conditions ordinarily classified as Hodgkin's disease belong to the reticulum cell group, either as reactive hyperplasias, aleukemic reticulocytomas or reticulum cell sarcomas.

FROM THE AUTHOR'S SUMMARY.

PRIMARY MALIGNANT MYXOMA OF THE HEART WITH METASTASES. E. FENSTER, Frankfurt. Ztschr. f. Path. **45**:565, 1933.

Forty-eight sarcomas of the heart, of which fourteen had produced metastatic tumors, have been reported in the literature. One additional case is reported, which showed the primary tumor in the right auricle in a 44 year old white man. Metastatic growths were found in the brain, suprarenal glands, renal capsules, liver and retroperitoneal and mesenteric lymph nodes.

H. HORN.

CARCINOMA AND RHINOSCLEROMA. G. GAEHTGENS, Frankfurt, Ztschr. f. Path. **46**:1, 1933.

A 63 year old man who had rhinoscleroma over a period of at least six years died of a primary squamous cell carcinoma of the nasal cavity, with metastases to various organs. The rhinoscleroma had been regarded as cured by x-rays. The belief is expressed that the carcinoma arose on the basis of rhinoscleroma, and that the marked metaplasia was one of the factors in the development of the carcinoma.

O. SAPHIR.

NEUROFIBROMATOSIS OF THE SYMPATHETIC NERVES, SPINAL CORD, BRAIN AND HYPOPHYSIS. L. MARTZ, Frankfurt. Ztschr. f. Path. **46**:119, 1933.

A case is described, and the related literature is reviewed. The entire sympathetic peripheral nervous system was involved, particularly the skin, the gastrointestinal tract and the suprarenal medulla. There also was a gliosis of the brain, spinal cord and posterior lobe of the hypophysis.

O. SAPHIR.

THE MESENCHYMAL TISSUE OF GLIOMAS. H. J. SCHERER, Virchows Arch. f. path. Anat. **291**:321, 1933.

Three types of mesenchymal tissue in gliomas are the essential stroma of the tumor, the reactive connective tissue laid down as the result of regressive changes in the tumor and the proliferated blood vessels.

O. T. SCHULTZ.

ORIGIN OF MIXED TUMORS OF THE LUNG. ANNELIESE MÖLLER, Virchows Arch. f. path. Anat. **291**:479, 1933.

A mixed tumor the size of a hazelnut, an incidental finding at necropsy in the lung of a man aged 44 years, is made the basis of a discussion of the possible origin of such tumors. The mesenchymal elements were fibrous, adipose and mucoid tissue; the epithelial constituent was columnar epithelium. In its architecture the tumor resembled an intracanalicular fibro-adenoma; it is termed a papillary tubular adenoma in which the formation of intracanalicular papillae with subsequent overgrowth of the papillary stroma was the essential and determining factor. The tumor is believed to have arisen from a bronchus and to have retained the developmental potencies of bronchi. The growth was inverted, that is intracanalicular, rather than everted, with the formation of the branching tubules of the developing bronchial system. Mixed tumors of this kind are held to arise from organ parts already well advanced in their embryogenesis.

O. T. SCHULTZ.

THE ORIGIN OF ORGANOID MIXED TUMORS. IRMGARD SCHMIDT, Virchows Arch. f. path. Anat. **291**:490, 1933.

Two cartilage-containing mammary tumors of the dog, with the general architecture of intracanalicular adenofibroma, furnish the foundation for views identical with those of Anneliese Möller in the preceding article.

O. T. SCHULTZ.

THE METHODS OF SEROLOGIC DIAGNOSIS OF CANCER BY MEANS OF LIPOIDAL FLOCCULATION. HERMANN LEHMANN-FACIUS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **80**:181, 1933.

The article summarizes the various modifications of the author's technic of cancer diagnosis. In all of them centrifugation of the serum-antigen mixtures was essential. The modifications were based on changes in the antigen and in the serum. The acetone-insoluble phosphatide fraction, the acetone-soluble fatty acid fraction and certain fatty acids of the primary alcoholic extracts were employed as antigens. The changes in the serum consisted in various modifications of the euglobulin fraction and in the treatment of the serum with various concentrations of hydrochloric acid. The best modification gave 80 per cent positive results with cancerous serums and no falsely positive results with the control serums, which included serums from pregnant women and from febrile and tuberculous patients. The substances in the cancerous serums, which participate in the reaction, were proved to be of antibody character because they could be separated from the antigen at 60 C.

I. DAVIDSOHN.

TUMOR IMMUNITY. HANS J. FUCHS and HUGON KOWARZYK, Ztschr. f. Immunitätsforsch. u. exper. Therap. **80**:375, 1933.

Fuchs and his associates reported in previous publications a test for the diagnosis of malignancy which is based on microchemical determination of nonprotein nitrogen in mixtures of serum or of serum fractions from patients with cancer with the serums of normal persons. Fuchs assumes the existence of an antigenic tumor-specific substance which is present in the circulation. It stimulates the production of antibodies, which may exist in the circulation alongside the antigen. In the present test both authors inoculated themselves with the serum from patients with cancer and observed the development of cancer antibodies, which could be determined by means of the microchemical test. Their serum behaved much as did the serums of patients who had antibodies against cancer either spontaneously or following irradiation or operation.

I. DAVIDSOHN.

TUMOR IMMUNITY. HANS J. FUCHS and M. VON FALKENHAUSEN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **80**:390, 1933.

The coexistence of the cancerous antigenic substance and of the antibody in the serum was demonstrated. The former was isolated in the albumin fraction, the latter in the globulin fraction of the serum.

I. DAVIDSOHN.

THE SEROLOGIC RELATIONSHIP BETWEEN FETAL BLOOD AND THE BLOOD OF PATIENTS WITH CANCER. HELLA AURIN, HUGON KOWARZYK and HANS J. FUCHS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **80**:420, 1933.

Aurin and his associates employed the Fuchs micro-method of determining the changes in the level of nonprotein nitrogen in mixtures of serum of one individual with certain fractions of the serum of another individual. They found that the serum of rabbit and human fetuses contained a substance which was absent in the maternal blood. The amount of this substance was highest in the early months of pregnancy, and it was absent at the end of gestation. From the absence of an

interaction between the fetal blood and the blood of patients with cancers, the authors assume a common factor in the embryonal and in the cancerous cell.

I. DAVIDSOHN.

THE HISTOLOGIC CRITERIA OF MALIGNANCY. M. BORST, Ztschr. f. Krebsforsch. **40**:3, 1933.

Borst considers the most essential criterion of malignant growth to be the demonstrable presence of locally destructive infiltration; even cellular displacement, as from metastasis, is insufficient if the other is absent. As regards individual cells, there is no general morphologic specific feature; the change from a benign to a malignant cell appears to be a gradual one. The presence of nuclear and particularly mitotic irregularity and evidence of unequal development of the tumor cells are of considerable, but not conclusive, diagnostic value. The relations of the tumor cells to their stroma are less often of considerable significance. Borst finds numerous objections to the grading of tumors on a histologic basis; reliability in the diagnosis and prognosis of tumor depends on experience and on knowledge of the special features of the part or organ involved. The histologic diagnosis should always be checked by the clinical and anatomic findings. While in many cases it is possible to make a diagnosis of malignant growth even in the absence of demonstrable infiltrative growth, there will always remain cases in which diagnosis on entirely histologic grounds will be uncertain.

H. E. EGGERS.

NEUROFIBROMATOSIS AND ITS RELATIONS TO GLIOMAS AND HERNIAS OF THE BRAIN. E. SCHAIRER, Ztschr. f. Krebsforsch. **40**:30, 1933.

Schairer reports here two cases of neurofibromatosis, both with associated changes in other organs. The first showed, in addition to widely scattered neurofibromas, a glioma of the aqueduct of Sylvius, two gliomatous nodules in the fourth ventricle and aqueduct, multiple hernias of the brain and tumors containing cerebral elements in the ethmoid. Schairer regards all of these abnormalities as of developmental origin. The second case showed an isolated neurofibroma of the vagus in the mediastinum. Here there were multiple mixed tumors of the kidneys, like those seen so frequently along with tuberous cerebral sclerosis, and Schairer considers that there is a similar relationship here.

H. E. EGGERS.

PERSISTENT ADIPOSITY IN CANCER. M. PRATES, Ztschr. f. Krebsforsch. **40**:71, 1933.

The occasional persistence of adiposity seen in cancerous persons may depend on a number of factors, some of which cannot be evaluated. While alteration of internal secretory functions may at times be responsible, this is certainly not constantly the case. Nor can it be ascribed to anemia, since this is most usually a feature of cachectic cases. Mostly it appears to depend on the location of the cancer and of its metastases. It most frequently occurs in the absence of involvement of the alimentary tract, especially the liver. In women, the climacteric predisposes to retention of body fat despite cancer. The morphologic study of the ductless glands in association with cancer reveals nothing of constant character. Prates observed in a fairly large proportion (44 of 175 cases) hypertrophy of the insular tissue of the pancreas. If endocrine changes are responsible, they must be of biologic character, not associated with morphologic change.

H. E. EGGERS.

Society Transactions

CHICAGO PATHOLOGICAL SOCIETY

Regular Monthly Meeting, Oct. 8, 1934

I. Pilot, *President, in the Chair*

Edwin F. Hirsch, *Secretary*

Presidential Address: Hemolytic Streptococci—Their Present Status and Their Relationhsip to Certain Clinical Entities. I. Pilot.

Hemolytic streptococci continue to be an important group of organisms about which there is considerable interest, and investigations are in progress with reference to their distribution, classification, differentiation and cultural variations. Such entities as scarlet fever, erysipelas and sporadic and epidemic septic sore throat are due to these streptococci. Their persistence in the foci of infection may explain the development of such sequelae as arthritis, glomerular nephritis and erythema nodosum.

The introduction of the fibrinolytic test and the rare sugars, trehalose and sorbite, have aided greatly in differentiating bovine from human strains. This identification is important in connection with the recognition of the state of carrier of hemolytic streptococci from bovine mastitis in milk which may be a source of epidemic septic sore throat.

Hemolytic streptococci of human origin have been divided largely on the basis of their origin into Str. scarlatinae, Str. erysipelatis and Str. epidemicus of septic sore throat. From cultural characteristics alone, the identification of these types is not possible, with the exception perhaps of Str. epidemicus. On ascites blood agar, this organism consistently forms large mucoid colonies with encapsulated cocci. However, a few of the scarlet fever and erysipelas strains may assume this mucoid character. The chocolate medium of Tunnicliff is of aid. In the experience of some investigators, the streptococci can be differentiated by agglutination and opsonic tests with specific antiserums. Toxin differentiation is perhaps most reliable. The toxin of scarlet fever appears to be specific and can be shown to be different from the toxin of septic sore throat and that of erysipelas by intradermal tests and tests for neutralization by specific antitoxic serums. A study of variations in this group indicates that the mucoid forms like Str. epidemicus may represent a variant with peculiarly aggressive properties such as are observed in other encapsulated organisms. Strains may lose their hemolytic power but still retain the capacity to produce specific toxin, as occurs in scarlet fever streptococci. The change of a hemolytic strain to one of nonhemolytic or viridans type appears to be due to a temporary loss of hemolytic property which can be restored by animal passage.

Sore throat in a patient reacting positively to scarlet fever toxin (Dick test), provided the causative streptococcus yields the specific toxin, results in scarlet fever. Sore throat without rash also may be due to the streptococcus of scarlet fever, to that of erysipelas or to Str. epidemicus. The great frequency of erysipelas on the face suggests that the nose and throat are the source of the infecting streptococci. In patients contracting otitis media, mastoiditis or cervical adenitis, the responsible cocci can be demonstrated in the throat. The persistence of these streptococci within the tonsils or other lymphoid structures of the pharynx leads to a state of carrier with these structures as foci of infection. Apparently, after an interval, the body may become sensitized to the streptococci. The acute polyarthritis observed after sore throat is often of a rheumatic type and may be the result of a

hyperergic reaction of joint tissue to the hemolytic streptococci or their products from the oral foci. A similar sensitivity of the skin may lead to erythema nodosum; of the kidney, to glomerular nephritis; of the endocardium, to endocarditis. Often it is possible to demonstrate the appearance and disappearance of the hemolytic streptococci in throat cultures simultaneously with the development and subsidence of these complications. The state of carrier of hemolytic streptococci following tonsillitis can be terminated by removing the faucial tonsils. The streptococci disappear in from one to twenty days. As patients with arthritis are often relieved by this procedure, the evidence points strongly to the streptococci as important etiologic factors in certain chronic forms of arthritis.

A Case of Dwarfism Associated with Lingual Goiter and Cystic Hypophysis. H. Gideon Wells.

This article will be published in full in a later issue of the Archives of Pathology.

Generalized Osteosclerosis with Chronic Polycythemia Vera. Edwin F. Hirsch.

This article appears in full in this issue, p. 91.

Effect of Atherosclerotic Plaques on the Diameter of the Lumen of Coronary Arteries. James D. Stewart, Eugene Birchwood and H. Gideon Wells.

A small series of hearts has been examined to determine the relation between the size of the lumen of the coronary artery at the site of atherosclerotic plaques as seen in the collapsed artery in the usual postmortem examination and the true size of the lumen when the artery is distended by the usual blood pressure. The results of this preliminary study indicate that a coronary artery exhibiting many atherosclerotic plaques which, as seen at postmortem examination, seem to cause marked local constrictions may, when distended by the usual blood pressure, possess a fairly uniform lumen without evidence of constriction. Apparently atherosclerotic plaques in a coronary artery do not necessarily protrude into the lumen during life, and the apparent narrowing seen in the dead body may not have existed during life.

Regular Monthly Meeting, Nov. 12, 1934

I. Pilot, *President, in the Chair*

Tumors of Islet Tissue with Hyperinsulinism in a Dog. Maud Slye and H. Gideon Wells.

This article will be published in full in a subsequent issue of the Archives.

Effects of Specific Immunization and of an Acacia Medium on Localization of Type I Pneumococci in Mice. Lloyd Catron.

Morphologic studies were made of the abdominal walls of young adult male white mice killed at intervals of from five minutes to five or six days after subcutaneous infection with living type I pneumococci. In normal mice, the bacteria disseminated readily through the subcutis and adjacent tissues, causing cellulitis and death of the host, the latter usually within forty-eight hours. There was no tendency to clumping of the organisms, and phagocytosis was negligible. To determine whether hyperergy or hypersensitiveness is an integral or essential part of immunity, two groups of mice had been specificially immunized, one actively and the other passively, by intraperitoneal injections of vaccine that had been

treated with dilute solution of formaldehyde and by injections of immune serum (rabbit); a third group was made both immune and hyperergic by intraperitoneal and repeated local vaccinations. In the first of these groups, as a result of vaccination, mononuclear phagocytes were mobilized in the subcutis. These cells ingested the subsequently injected pneumococci as early as five minutes after infection; the bacteria were localized and eradicated in the presence of the least amount of exudate, edema and necrosis of any of the groups studied. After passive immunization, the bacteria were immediately clumped in the tissues, soon ingested by mononuclear cells and granulocytes, and destroyed in the presence of sharply circumscribed small abscesses in the subcutis. In the group made hyperergic as well as immune, immediate phagocytosis by mononuclear cells occurred. The more extensive edema, exudation, necrosis and abscess formation resulting from the local vaccinations seemed to be not an essential factor in immunity, since in their absence there was equally effective localization and destruction of the bacteria. Since an acacia medium has been reported both to enhance and to delay immunoagglutination in vitro, presumably as a result of its high viscosity, the effects of acacia inocula with or without a trace of immune serum were studied. When pneumococci were suspended in a 10 per cent solution of acacia in saline solution and injected into normal mice, the organisms were localized to the site of injection for one hour, with formation of small clumps. The acacia also hindered the ingress of host cells, however, and the bacteria soon spread throughout the abdominal wall, causing a more extensive and destructive lesion and earlier death of the host than when a saline medium was used. In the presence of 0.001 cc. of immune serum, on the other hand, much better localization was maintained with acacia than with a saline medium. Access of phagocytic cells to the bacteria was delayed as with acacia alone, but at four and more hours after infection there were large clumps of cocci in the subcutis, and phagocytosis was extensive; at late stages, sharply circumscribed small abscesses were confined to the subcutis.

Under the experimental conditions, immunity was not dependent on hyperergy or hypersensitiveness. In vivo agglomeration localized the pneumococci for a time, during which they continued to proliferate; their destruction depended on phagocytosis. Macrophages or mononuclear forms played a much more significant part in phagocytosis than did granulocytes. The single factor of chief importance in localizing the pneumococcus was specific antibody. An acacia medium, after temporarily localizing the bacteria, ultimately caused a lesion much more severe than that obtained with a saline inoculum; this relationship, however, was reversed in the presence of a small amount of immune serum, far greater bacterial localization and destruction being maintained with the more viscous medium.

DISCUSSION

P. R. CANNON: There is a similarity between these experiments and those of Gay and his associates with hemolytic streptococci. They noted that various irritants injected into the pleural space of rabbits caused exudates either of polymorphonuclear leukocytes or of mononuclear phagocytes (clasmatocytes). When virulent hemolytic streptococci were injected into a pleural exudate of polymorphonuclear leukocytes, the animals died, whereas with the mononuclear type of exudate the animals could withstand several hundred times the lethal dose. When Gay applied this to pneumococci, however, the same relation did not hold. The presence of many mononuclear phagocytes did not localize the pneumococci, but they were localized by the presence of a small amount of specific immune serum. This emphasizes the efficacy of specific serums as contrasted with nonspecific serums. Many believed Krause and his associates, who from their work with tubercle bacilli stated that immunity is due to allergy. Rich showed that pneumococci injected into passively immunized animals deprived of their leukocytes at first are localized without the presence of leukocytes or fibrin. After twenty-four hours, of course, the pneumococci spread. The immediate localization represents an antigen-antibody reaction and not inflammation. The latter enters later and maintains the localization of the infection.

W. E. ADAMS: Reinhoff Jr. made clinical application of this principle for pneumonectomies and lobectomies. Beef broth injected intrapleurally produced a monocytic and fibrous exudate and consequent local immunity. The operative procedures then were accomplished with a remarkably low mortality.

S. ROSENBERG: When an exudate is alkaline in reaction the cells are predominately leukocytes; if acid, the cells are mononuclear phagocytes. Were changes in p_H factors in these experiments?

L. CATRON: No attempt was made to measure the p_H of these exudates.

LIPOID PNEUMONIA. PAUL R. CANNON.

Lipoid pneumonia is caused by aspiration of fats or oils into the lungs. It is seen most frequently in infants and children, but may occur at any age, particularly as a consequence of gavage in demented patients, or of cardiospasm and vomiting or unconsciousness and vomiting, or of the administration of mineral oil to dysphagic patients or of the nasopharyngeal use of oily sprays. Several writers have recently pointed out hazards in the use of "nose drops" containing various antiseptics in oil for the treatment of infections of the upper respiratory tract in infants and children, and in improper giving of cod liver oil, especially to infants.

Pinkerton, Laughlen, Pierson, Moore and Gross, and Fischer-Wasels and Rabinowitch and Lederer in recent papers described the types of oils and fats responsible, the pathogenesis of the pneumonia and the pathologic effects. It is probable that many cases of so-called "desquamative pneumonia" and "giant cell pneumonia" are instances of lipoid pneumonia. In the past, however, more attention has been given to the nature of the giant and mononuclear cells than to the causal factors in this pneumonia. The latter presents a variable picture, characterized usually by development of areas of consolidation, atelectasis, abscess formation, fibrosis, empyema and secondary infection. Vacuolated, lipoid-filled septal or other mononuclear cells, giant cells and granulation tissue constitute the outstanding microscopic picture.

Mineral oil, milk fat, egg yolk and various combinations of antiseptics in vegetable or mineral oils or cod liver oil have usually been responsible for the lipoid pneumonia. Pinkerton reported observing this condition in almost 2 per cent of infants and young children at routine necropsies, and both he and Laughlen emphasized the importance of the aspiration of oil sprays, nasal drops or cod liver oil in causing the pneumonia. I report an instance of aspiration of the latter because of its importance in pediatric practice.

A boy, 5 months of age, entered the hospital because of persistent cough and fever of about five weeks' duration. Examination suggested bronchopneumonia of the right upper pulmonary lobe. X-ray plates revealed several cavities in the right lung. The total duration of illness was approximately eight weeks, during which the condition became progressively worse, with development of generalized peritonitis a few days before death. The parents stated that the symptoms appeared shortly after administration of cod liver oil the first time, which was on the first day of his second month. Ten minims (0.6 cc.) were given by medicine dropper with the child on his back. The dose was gradually increased, but after two weeks the child refused to take the oil. Because of medical advice to continue the treatment, the parents forced him to take the oil although at times as many as five doses were given before he could be made to swallow one. In forcing him to take the oil, the parents on two occasions held his nose, but this was not done until after he had already coughed and choked when the oil was given. Several brands of cod liver oil were used, prepared by reputable pharmaceutic houses.

The necropsy revealed the following essential features: pneumonia due to aspiraton of lipoid, with extensive formation of abscesses in the right upper and lower lobes; focal fibrinous and fibrinopurulent pleuritis on the right side; acute generalized fibrinopurulent peritonitis, and suppurative otitis media on the left side. Postmortem cultures yielded Streptococcus haemolyticus from the peritoneal cavity, Str. haemolyticus and Bacillus coli from the pulmonary abscesses and Str. haemo-

lyticus, B. coli and Bacillus pyocyaneus from the right pleural cavity. Microscopic examination showed a desquamative pneumonia in which the alveolar spaces were filled with a yellowish lipoid, many vacuolated septal cells filled with lipoid, and only moderate numbers of polymorphonuclear leukocytes. Abscess cavities filled with lipoid, amorphous débris and leukocytes were surrounded by granulation tissue in which were occasional giant cells. Polymorphonuclear leukocytes were conspicuous mainly within the lumens of bronchi.

A detailed consideration of lipoid pneumonia is unnecessary in view of the excellent discussions of the subject available in the literature. The pathogenesis is the most important feature, and physicians should realize the dangers in improper administration of cod liver oil, oil sprays or oil nasal drops, especially when given to infants in a reclining position. Pinkerton has suggested that the blandness of oils and the anesthetizing action of menthol or other oils may facilitate their passage through the larynx because of a depression of the cough reflex. Walsh and I instilled a solution of trypan blue into the nostrils of rabbits and found that it reached the lungs directly through the trachea. I also instilled a few drops of a 50 per cent emulsion of cod liver oil into the nostrils of rabbits held in an erect position for approximately ten minutes and observed the oil in the lungs a few days later. Laughlen saw the same effect following a spraying of the noses and throats of rabbits with mineral oil.

DISCUSSION

F. BALL: Recently I had as a patient a man, 96 years of age, who for many years had used a medicated petrolatum oil spray for his nose and throat. Post-mortem examination demonstrated an extensive scarring of his lungs, which was due, as demonstrated by histologic studies, to quantities of aspirated oil. Old lesions were represented by fibrous scars containing droplets of oil; the more recent lesions, by exudates of swollen monocytes laden with finely divided oil.

J. LEWIS: Does iodized poppy-seed oil 40 per cent injected into the bronchi produce these changes?

O. SCHULTZ: In experiments of my acquaintance iodized poppy-seed oil did not cause inflammatory reactions. The discussion so far has been of oils inhaled into the lungs, but in certain disturbances of lipoid metabolism lipoid material may be introduced into the lungs through the circulation.

I. PILOT: Abscesses were produced in the lungs of animals experimentally when sputum was injected intratracheally with poppy-seed oil. Sputum alone did not produce such lesions. The oil enabled the bacteria to multiply.

W. E. ADAMS: Iodized poppy-seed oil introduced intratracheally into dogs produces local scarring. In man the oil remains for a considerable time. It is not known why there sometimes are inflammatory reactions and sometimes not.

E. F. HIRSCH: Oils remaining in tissues are solvents for lipoid substances such as cholesterol. Fatty acids in oil attract bases such as calcium or iron, and the presence of such compounds is important in determining whether the oil is innocuous or capable of stimulating an inflammatory reaction.

LESIONS INDUCED IN THE LUNGS BY INTRAVENOUS INJECTION OF TAR. JAMES P. SIMONDS and JOHN S. CURTIS.

This article will appear in full in a later issue of the ARCHIVES.

Book Reviews

Amebiasis and Amebic Dysentery. By Charles F. Craig, M.D., M.A. (Hon. Yale), F.A.C.P., F.A.C.S.; Colonel, United States Army, retired; D.S.M.; Professor of Tropical Medicine and Head of the Department of Tropical Medicine, Tulane University of Louisiana School of Medicine, New Orleans; formerly Commandant, Army Medical School and Director of the Department of Clinical Pathology and Preventive Medicine, and Assistant Commandant, Army Medical Center, Washington, D.C. Price, $5. Pp. 315, with 54 Illustrations. Springfield, Ill.: Charles C. Thomas, Publisher, 1934.

This book is based on a thorough study of the literature on amebiasis and the results derived from the authors' "own experience covering over thirty years of study of amebic infection from the laboratory, epidemiological and clinical standpoints." The book is opportune because the epidemic of amebic dysentery in Chicago in 1933, which gave rise to several hundred cases of the disease throughout the country, created an acute demand on the part of physicians for reliable information regarding amebic infection, which is known to be widespread. Colonel Craig's monograph meets well this demand. The double-headed title is used because "the term amebic dysentery is so generally thought to indicate a distinct disease and the term amebiasis is so seldom considered by physicians as including amebic dysentery." There are twelve chapters. In the first chapter the author defines amebiasis and amebic dysentery and sketches the history of the present knowledge of the infection and of its geographic distribution. The second chapter is devoted to the etiology and the life story and characteristics of Endamoeba histolytica, and the third chapter deals with the epidemiology of amebic infection. Chapters 4 and 5 describe the pathologic anatomy of amebiasis in all its forms and localizations. The distinctive features of the changes in the invaded tissues in pure amebic infection are described clearly, and the fact is emphasized strongly that in the so-called amebic carrier there are apparently always more or less well marked typical lesions in the mucous membrane of the large intestine. Pathologists should be on the lookout for instances of amebic appendicitis in the routine examination of appendixes. The next four chapters deal with the symptoms, complications, sequelae and diagnosis of amebiasis. In chapter 10 the author's complement-fixation test for amebiasis is described in detail. This test has been found "highly specific for amebic infection," and while limited in use by the difficulty of preparing good antigens it may be of great help in the diagnosis of the condition in suspected carriers and in cases in which the ameba cannot be demonstrated in the feces. In chapter 11 the author discusses the prognosis and prophylaxis of amebiasis, and in chapter 12 the treatment. At the end of each chapter is a list of the most important articles dealing with the topic under discussion. There are an index of authors and a good general index. The book is printed on rather heavy glazed paper. The type used for section headings in the text seems to be heavier and larger than necessary. There are fifty-four illustrations, mostly photomicrographs of the ameba. The style is clear and orderly. The presentation is competent and authoritative. In the development of the understanding of amebic infection and its importance to public health, as well as of its nature, diagnosis and treatment, the author has taken a significant part, and the book reflects well the state of knowledge of amebiasis in all its phases. It will be a landmark in its field.

Books Received

CLINICAL PATHOLOGY OF THE JAWS WITH A HISTOLOGIC AND ROENTGEN STUDY OF PRACTICAL CASES. Kurt T. Thoma, D.M.D., Charles A. Brackett Professor of Oral Pathology in Harvard University, Oral Surgeon to the Brooks Hospital, Consulting Oral Surgeon to the New England Baptist Hospital and Consulting Oral Surgeon to the Tumor Clinic of the Beth Israel Hospital. Price, $9. Pp. 643, with 423 illustrations. Springfield, Ill.: Charles C. Thomas, Publisher, 1934.

A TEXTBOOK OF BACTERIOLOGY WITH A SECTION ON PATHOGENIC PROTOZOA: THE APPLICATION OF BACTERIOLOGY AND IMMUNOLOGY TO THE ETIOLOGY, DIAGNOSIS, SPECIFIC THERAPY AND PREVENTION OF INFECTIOUS DISEASES FOR STUDENTS AND PRACTITIONERS OF MEDICINE AND PUBLIC HEALTH. Hans Zinsser, M.D., Professor of Bacteriology and Immunology, Harvard University Medical School, and Consulting Bacteriologist to the Peter Bent Brigham Hospital and the Children's Hospital, Boston, and Stanhope Bayne-Jones, M.D., Professor of Bacteriology, Yale Unversity Medical School, and Master of Trumbull College, Yale University. Edition 7, rewritten, revised and reset. Price, $8. Pp. 1,226, with 174 illustrations. New York: D. Appleton-Century Company, Inc., 1934.

A TEXTBOOK OF PATHOLOGY FOR NURSES. Coleman B. Rabin, B.S., M.D., Lecturer in Pathology in the Mount Sinai Hospital School of Nursing and Assistant in Morbid Anatomy, Adjunct Physician and Assistant Radiologist to the Mount Sinai Hospital, New York. Price, $1.75. Pp. 243, with 61 illustrations. Philadelphia: W. B. Saunders Company, 1934.

TESTS FOR RESPIRATORY EFFICIENCY. Alan Moncrieff. Medical Research Council, Special Report Series, No. 198. Price, 1 shilling. Pp. 62, with 7 illustrations. London: His Majesty's Stationery Office, 1934.

MANUAL OF CLINICAL LABORATORY METHODS. Pauline S. Dimmitt, Ph.G., Medical Technologist for the Stout Clinic, Sherman, Texas. Price, $2. Pp. 156, with 36 engravings, including 7 full-page colored plates. Philadelphia: F. A. Davis Company, 1934.